The Professional Medical Journal
www.theprofesional.com
DOI: 10.17957/TPMJ/15.3087

ORIGINAL PROF-3087

BREAST CANCER;
APOPTOSIS INHIBITORY PROTEIN SURVIVIN AND CASEIN KINASE 2

1. MBBS, M Phil, Assistant Professor, Department of Biochemistry & Molecular Biology Army Medical College, National University of Sciences & Technology, Abid Majeed Road, Rawalpindi.
2. MBBS, M Phil, FCPS, PhD, Professor, Department of Biochemistry, Islamic International Medical College, Riphah International University, Islamabad.
3. MBBS, FCPS, Histopathologist, Department of Pathology, Army Medical College, Rawalpindi.
4. MBBS, MPhil, Department of Pathology, Army Medical College, Rawalpindi.

Correspondence Address:
Dr. Sarah Sadiq
MBBS, M Phil, Assistant Professor, Department of Biochemistry & Molecular Biology Army Medical College,
National University of Sciences & Technology,
Abid Majeed Road, Rawalpindi.
sarahsadiq10@hotmail.co.uk

Article received on:
08/09/2015
Accepted for publication:
15/10/2015
Received after proof reading:
00/00/0000

Dr. Sarah Sadiq[1], Dr. Abdul Khaliq Naveed[2], Dr. Shahid Jamal[3], Dr. Aiza Sadia[4]

ABSTRACT...: CK2 enzyme is up regulated in several cancers. It has many substrates including survivin which is up regulated in cancers. **Objectives:** To find out the correlation between expression of CK2α and survivin and evaluate it as a prospective prognostic marker in pathogenesis of the breast cancer and to find if positive correlation between CK2 and survivin was associated with advancing disease. **Study Design:** Cross Sectional Analytical type of study. **Setting:** Department of Biochemistry & Molecular Biology, Army Medical College, Rawalpindi and Armed Forces Institute of Pathology, Rawalpindi. **Duration of study**: January 2013-December 2014. **Methods:** The research protocol was approved by Armed Forces Institute of Pathology Ethical Committee. Paraffin embedded tissue sections of diagnosed breast cancer, obtained from AFIP, were used. Immunohistochemistry was performed to determine nuclear and cytoplasm expression of survivin, and CK2 .Scoring done by three histopathologists, independently. **Results:** Total CK2 expression was high in invasive as compared to non-invasive cases (p =0.209). Cytoplasm and nuclear localization of CK2 in invasive group was a little higher too (p = 0.092) and (p=0.286) respectively. Total survivin expression was high in invasive as compared to non-invasive cases (p= 0.449). Cytoplasm and nuclear localization of survivin in invasive group was higher as compared to noninvasive group with no significant different (p=0.472) and (p=0.367) respectively. A positive and strong correlation was found in CK2 and survivin expression and localization in both non-invasive as well as invasive groups. **Conclusion:** CK2 and survivin correlation in cancers can be used in predicting the cancer phenotype and aggression at early stages.

Key words: CK2α, survivin, Ca Breast, Immunohistochemistry

Article Citation: Sadiq S, Naveed AK, Jamal S, Sadia A. Breast cancer; apoptosis inhibitory protein survivin and casein kinase 2. Professional Med J 2015;22(12):1595-1600. **DOI:** 10.17957/TPMJ/15.3087

INTRODUCTION

The most prevalent cancer, among women is Breast cancer, causing death of over 508 000 women in 2011.[1] Tumor markers are significant in the breast cancer research because of their influence on the prognosis.[2]

CK2 is present in eukaryotic cells, having more than 100 substrates.[3] CK2 is up-regulated in cancers.[4] Is a ubiquitous serine / threonine kinase[5] Its elevated expression in tumors makes it a candidate for molecular-targeted therapy.[6]

Apoptotic deregulation leads to pathology.[7] Apoptotic inhibitory family (IAPs) target mainly caspase 3 and 7.[8] The genetic evidence to classify IAPs as oncogenes is IAP gene amplification[9] More efforts are needed to find strategies that can target these proteins.[10] The most convincing evidence for the IAP involvement in cancers is seen by IAP named survivin.[8] Survivin (BIRC5), has high expression in cancers and is connected with poor outcome clinically.[10] The smallest member of IAP family having a 16.5kDa protein weight consisting of 142-aminoacids, is encoded by gene on the human 17q25 chromosome,.[11] Exists as a homodimer which is functional.[12] Is implicated in controlling cell survival and regulating mitosis in cancers.[13] Survivin is a substrate of CK2.[22]

MATERIAL AND METHODS

Paraffin embedded tissue sections of breast cancer (N=30) diagnosed by pathologist were selected, in Armed Forces Institute Of Pathology Rawalpindi Pakistan. All experiments were repeated twice.

MATERIALS AND CHEMICALS

The Case in Kinase IIα, Antibody (C-18); at polyclonal, Ig G 200μg/ml from Santa Cruz,(cat#6479), was used, with 1:200 dilution, using Hela and the Jurkat cell lysate as positive control. Monoclonal Mouse Anti-Human Survivin Clone 12C4: Dako (Ref) M3624, 1:100, the Detection Kit: LSAB Kit/ HRP, Rb/ Mo/ Goat, (DAB+) system from (DAKO) (Ref:K0679). the Antibody Diluting Reagent Solution:(Ready to Use), from Invitrogen Ref NO 003218 (contains 0.1% Sodium Azide).

IMMUNOHISTOCHEMISTRY

Tissue sections from paraffin blocks, thickness 3-4 microns, kept at (40˚C-45˚C) in water bath, shifted to slides and kept in oven for 2 hrs at 56˚C. Slides deparaffinized with absolute xylene, absolute alcohol, then 80% alcohol, the in 70% alcohol and finally dipped in the water. Antigen retrieval by treating with 10X EDTA + TRIS Antigen Retrieval Solution at 100□C in the electric decloaking chamber, for 25 minutes. Washed with distilled water, then Phosphate Buffer Solution, then blocked, using Peroxidase Blocking Solution, S2023 DAKO. Washed with PBS thrice. Incubated with Primary Antibody for one hour, washed with PBS, then secondary antibodies treatment for 15 minutes followed by washing. Slides were then treated with Streptavidin-HRP, 15 minutes, washed and DAB Chromogen was spread for 10 minutes. Washing with the distilled water, and counterstaining done with Haematoxylin, followed by washing thrice Slides treated with the alcohol 90%, 80% and 70% and Xylene 90%, 80% and 70%. Mounted with DPX coated coverslips.

Scoring was done, by 3 histopathologists, independently and any divergence in results was amended, using the nearest readings.

CK2 scoring was: 0 = no stain, 1+ = weakly stained, 2+ = moderately stained, 3+ = strong staining. Nucleus and cytoplasm scoring was done, sum of both scores = total expression levels of CK2. Survivin scoring done as: the percentage of positive cells: 1. 1-10%, 2; 11-50%, 3. 51-80%, 4; >80% positive cells. Staining intensity as 1, weak; 2, moderate, 3, intensive. The scores of positive cells and the scores of expression intensities were then multiplied to find the immunoreactive score (IRS), 0-2 = no stain; 3-4 = weak stain, 6-8 = moderate stain; 9-12 = strong staining.

STATISTICAL ANALYSIS

Data had been analyzed by using statistical software SPSS version 20. The analysis was carried out on CK2 nuclear, cytoplasm and total expression and survivin nuclear, cytoplasm and immunoreactive scores, in invasive and non-invasive groups separately. Descriptive statistics was calculated by using Mean±SD. Independent sample t-test was used to compare quantitative variables, in groups. Correlation analysis was performed to determine the association between survivin and CK2, in invasive and non-invasive groups separately. A p-value less than 0.05 (two-sided) was considered to be statistically significant.

RESULTS

Total thirty cases of diagnosed breast cancer (invasive ductal carcinoma) were included.

Per neural invasion was present in 20(66.7%) patients and absent in 10(33.3%) patients. Average Nottingham index mean score in the invasive cases was 6.0±1.16691, in noninvasive cases was 4.06±0.75011. Total CK2 expression was high in invasive as compared to non-invasive cases.

Cytoplasm localization of CK2 in invasive group was higher as compared to noninvasive group. Nuclear localization was also not statistically significant between the groups with higher in invasive cases than non-invasive. Total survivin expression was high in invasive in comparison to non-invasive cases. The difference was insignificant between the two groups. Cytoplasm localization of survivin in invasive group was higher as compared to noninvasive group. Nuclear localization of survivin was insignificantly different between the groups with higher in invasive cases than non-invasive (Table-I).

Characteristics	Groups	Mean ± S.D	p-value
CK2Nuc	Non-Invasive	1.4±0.52	0.092
	Invasive	1.9±0.85	
CK2Cyto	Non-Invasive	0.80±0.63	0.286
	Invasive	0.95±0.83	
CK2Total	Non-Invasive	2.2±1.03	0.209
	Invasive	2.8±1.3	
survivin Cyto	Non-Invasive	6.6±3.5	0.472
	Invasive	7.7±3.9	
survivin Nuc	Non-Invasive	4.6±2.5	0.367
	Invasive	5.5±3.03	
survivin total	Non-Invasive	11.2±5.26	0.449
	Invasive	13.2±6.22	

Table-I. Mean Comparison of characteristics between groups

A positively strong correlation was found in CK2 expression and localization and survivin expression and localization in both non-invasive as well as invasive groups. In non-invasive cases, moderate correlation was seen between survivin and CK2 in nucleus. Very strong and positive correlation existed between total survivin and CK2 expression in nucleus, cytoplasm and total CK2 expression. Similarly very strongly positive correlation was found between total CK2 and total survivin including nuclear as well as cytoplasm survivin level (Table-II.I).

In invasive cases, CK2 nuclear expression was strongly correlated with survivin expression in nucleus as well in cytoplasm. Strong correlation was also seen between CK2 total expression and survivin cytoplasm expression and between total survivin and CK2 levels (Table-II.II).

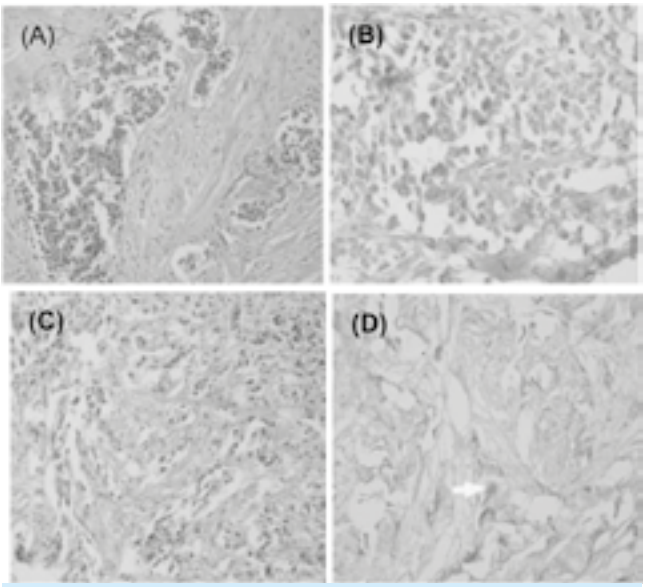

Figure. Photomicrograph of breast carcinoma tissue specimen. *(A) Representative H&E (B) Immune staining of CK2α showing +3 nuclear, 0 cytoplasm staining of CK2α scores. (C) Representative H&E figure (D) Showing +8 nuclear and + 12 cytoplasm staining of survivin antibody.*

Characteristics		Survivin Nuc	Survivin Cyt	Survivin Total
CK2_Nuc	Pearson Correlation(r)	0.653*	0.601	0.825**
	P-value	0.041	0.066	0.003
CK2_Cyt	Pearson Correlation(r)	-	-	0.919**
	P-value	-	-	0.001
CK2_Total	Pearson Correlation(r)	0.875**	0.919**	0.727*
	P-value	0.001	0.001	0.017

Table-II.I. Correlation analysis in Non-invasive cases

Characteristics		Survivin Nuc	Survivin Cyt	Survivin Total
CK2_Nuc	Pearson Correlation(r)	0.570**	0.536*	0.620**
	P-value	0.009	0.015	0.004
CK2_Cyt	Pearson Correlation(r)	0.116	-	0.310
	P-value	0.628	-	0.184
CK2_Total	Pearson Correlation(r)	0.419	0.566**	0.565**
	P-value	0.066	0.009	0.009

Table-II.II. Correlation analysis in Invasive cases

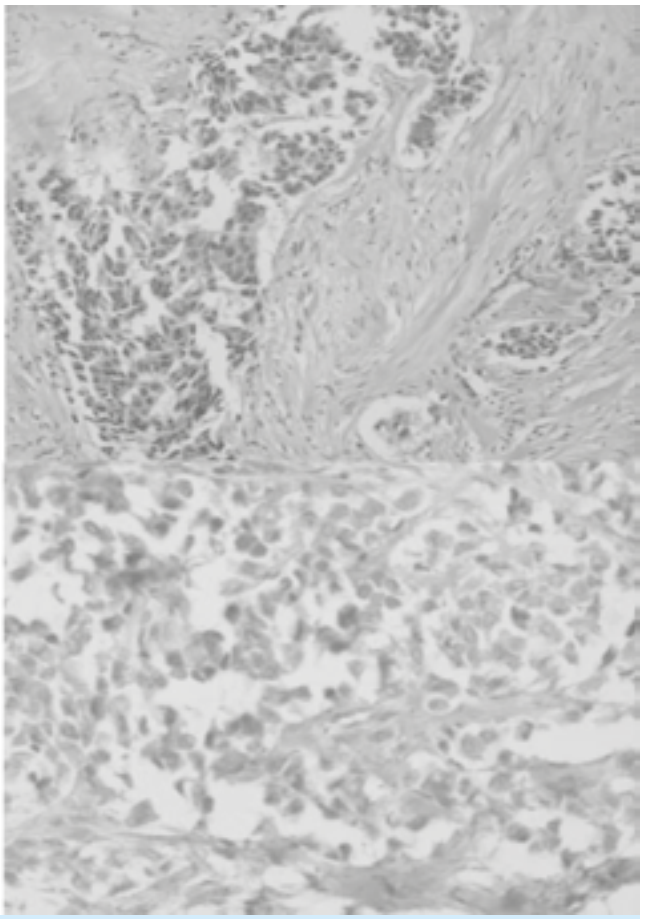

Fig-1. Represents H&E and weak cytoplasmic and strong nuclear staining for CK2α

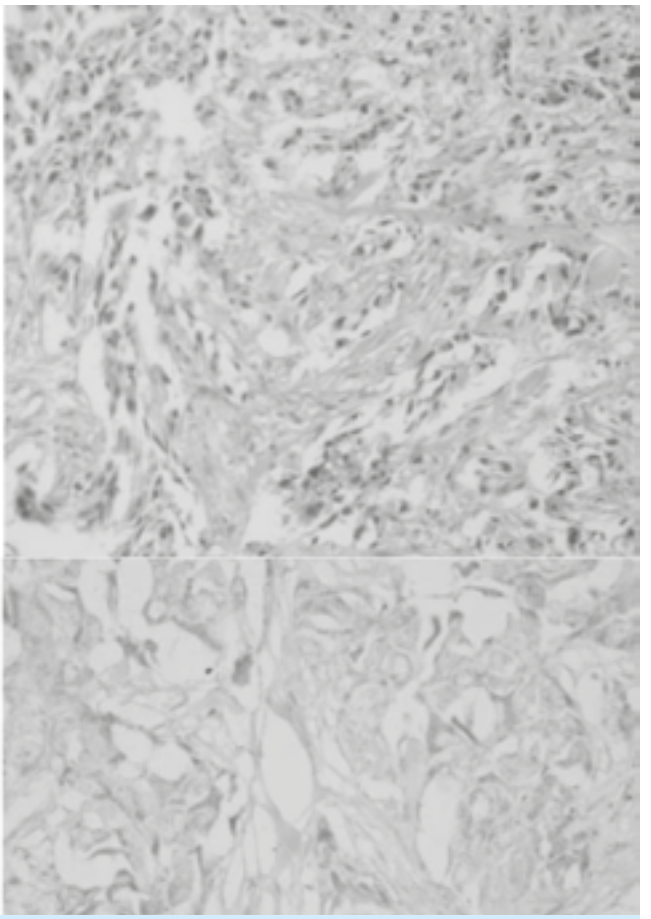

Fig-2. Showing H&E and strong survivin antibody staining

DISCUSSION

There is sufficient substantiation that CK2 is over expressed in the proliferative states. Experimental studies by Tawfic et al show that deregulated expression of a subunit of CK2 indicates oncogenic potential in cells so that in collaboration with some oncogenes it causes a reflective enhancement of tumor phenotype.[14] CK2, is a known pleiotropic serine/threonine protein kinase[15], participates in an array of cellular processes targeting more than 300 substrates[16]. CK2 expression is reported to be elevated in human cancers[17], but how this up-regulations plays role in carcinogenesis is yet to be cleared[18]. It has been established that a large variety of different types of cancer cells depend on raised CK2 level for their continued existence[19], keeping this in view, we investigated the co-expression pattern evaluation of CK2α and survivin in the breast cancer. We found a positive correlation of these proteins in breast cancerous tissues, when immunohistochemically stained.

Previously, many people have reported independent over expression of CK2 and survivin in breast cancer. Lu et al showed that survivin levels were raised in ErbB2-overexpressing cells in many breast cancer patient samples as well as in cell lines ,showing resistance to Taxol drug induced apoptosis[20] It was demonstrated by Yde et al that CK2 inhibition by CK2 specific inhibitor, 2-dimethylamino-4,5,6,7-tetrabromo-benzimidazole (DMAT), leads to caspase-mediated death of tumor cells in human breast carcinoma[21] Tapia et al observed that TBB decreases Survivin Levels in breast and colorectal cancer cells, HT29 (US) colon cancer cells.

Comparable results were seen in human DLD-1 and SW-480 colorectal, and ZR-75 breast cancer and HEK-293T embryonic kidney cells suggesting that CK2 may be regulating the expression of survivin.[22] Our work was in consistency with the previous work.

CK2α over expression has been correlated with survivin expression previously.[23] CK2 modulates apoptotic activity via IAPs (apoptosis inhibitory proteins)[24] and it has been observed that CK2-mediated survivin up-regulation leads to enhanced cell survival and tumor genesis.[25] In prostate cancer cells CK2 inhibition study has also proved the link between CK2 expression and survivin as inhibiting CK2 also led to decrease of survivin.[26]

ACKNOWLEDGEMENT

I acknowledge support from Commandant Armed Forces Institute of Pathology Major General Muhammad Ayyub and Head of Surgical Department, CMH Rawalpindi, Major General Maqbool Ahmed, Department of Histopathology, AFIP and Army Medical College, for the study.

CONCLUSION

There is a positive and moderate correlation between survivin and CK2α in the pathogenesis of breast cancer. Combined expression of CK2α and survivin can be used as biomarkers for predicting the cancer phenotype and aggressiveness.

REFERENCES

1. Organization WH. **Global Health Estimates Summary Tables: Dalys by cause, age and sex.** Geneva: WHO. 2013.

2. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. **Descriptive analysis of estrogen receptor (ER)‐negative, progesterone receptor (PR)‐negative, and HER2‐negative invasive breast cancer, the so‐called triple‐negative phenotype.** Cancer. 2007;109(9):1721-8.

3. Allende J, Allende C. Protein kinases. 4. **Protein kinase CK2: an enzyme with multiple substrates and a puzzling regulation.** The FASEB journal. 1995;9(5):313-23.

4. Götz C, Gratz A, Kucklaender U, Jose J. **TF—A novel cell-permeable and selective inhibitor of human protein kinase CK2 induces apoptosis in the prostate cancer cell line LNCaP.** Biochimica et Biophysica Acta (BBA)-General Subjects. 2012;1820(7):970-7.

5. Brown MS, Diallo OT, Hu M, Ehsanian R, Yang X, Arun P, et al. **CK2 modulation of NF-kappaB, TP53, and the malignant phenotype in head and neck cancer by anti-CK2 oligonucleotides in vitro or in vivo via sub-50-nm nanocapsules.** Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(8):2295-307.

6. Turowec JP, Duncan JS, French AC, Gyenis L, St Denis NA, Vilk G, et al. **Protein kinase CK2 is a constitutively active enzyme that promotes cell survival: strategies to identify CK2 substrates and manipulate its activity in mammalian cells.** Methods Enzymol. 2010;484:471-93.

7. Ola MS, Nawaz M, Ahsan H. **Role of Bcl-2 family proteins and caspases in the regulation of apoptosis.** Mol Cell Biochem. 2011;351(1-2):41-58.

8. LaCasse EC, Baird S, Korneluk RG, MacKenzie AE. **The inhibitors of apoptosis (IAPs) and their emerging role in cancer.** Oncogene. 1998;17(25):3247-59.

9. Liston P, Fong WG, Korneluk RG. **The inhibitors of apoptosis: there is more to life than Bcl2. Oncogene.** 2003;22(53):8568-80.

10. Kelly RJ, Lopez-Chavez A, Citrin D, Janik JE, Morris JC. **Impacting tumor cell-fate by targeting the inhibitor of apoptosis protein survivin.** Mol Cancer. 2011;10(35):10.1186.

11. Li F, Altieri DC. **The Cancer Antiapoptosis Mouse Survivin Gene Characterization of Locus and Transcriptional Requirements of Basal and Cell Cycle-dependent Expression.** Cancer research. 1999;59(13):3143-51.

12. Chantalat L, Skoufias DA, Kleman J-P, Jung B, Dideberg O, Margolis RL. **Crystal structure of human survivin reveals a bow tie–shaped dimer with two unusual α-helical extensions.** Molecular cell. 2000;6(1):183-9.

13. Blanc-Brude OP, Mesri M, Wall NR, Plescia J, Dohi T, Altieri DC. **Therapeutic Targeting of the Survivin Pathway in Cancer Initiation of Mitochondrial Apoptosis and Suppression of Tumor-associated Angiogenesis.** Clinical Cancer Research. 2003;9(7):2683-92.

14. Tawfic S, Yu S, Wang H, Faust R, Davis A, Ahmed K. **Protein kinase CK2 signal in neoplasia.** 2001.

15. Dastidar EG, Dayer G, Holland ZM, Dorin-Semblat D, Claes A, Chêne A, et al. **Involvement of Plasmodium**

falciparum protein kinase CK2 in the chromatin assembly pathway. BMC biology. 2012;10(1):5.

16. Yaylim I, Ozkan NE, Isitmangil T, Isitmangil G, Turna A, Isbir T. **CK2 enzyme affinity against c-myc 424-434 substrate in human lung cancer tissue.** Asian Pacific journal of cancer prevention : APJCP. 2012;13(10):5233-6.

17. Channavajhala P, Seldin DC. **Functional interaction of protein kinase CK2 and c-Myc in lymphomagenesis.** Oncogene. 2002;21(34):5280-8.

18. Pornchai O, Rusch V, Talbot SG, Sarkaria I, Viale A, Socci N, et al. **Casein kinase II alpha subunit and C1-inhibitor are independent predictors of outcome in patients with squamous cell carcinoma of the lung.** Clinical cancer research. 2004;10(17):5792-803.

19. Ruzzene M, Pinna LA. Addiction to protein kinase **CK2: a common denominator of diverse cancer cells? Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics.** 2010;1804(3):499-504.

20. Lu J, Tan M, Huang W-C, Li P, Guo H, Tseng L-M, et al. **Mitotic deregulation by survivin in ErbB2-overexpressing breast cancer cells contributes to Taxol resistance.** Clinical Cancer Research. 2009;15(4):1326-34.

21. Yde CW, Frogne T, Lykkesfeldt AE, Fichtner I, Issinger O-G, Stenvang J. **Induction of cell death in antiestrogen resistant human breast cancer cells by the protein kinase CK2 inhibitor DMAT.** Cancer letters. 2007;256(2):229-37.

22. Tapia J, Torres V, Rodriguez D, Leyton L, Quest AF. **Casein kinase 2 (CK2) increases survivin expression via enhanced β-catenin–T cell factor/lymphoid enhancer binding factor-dependent transcription.** Proceedings of the National Academy of Sciences. 2006;103(41):15079-84.

23. Ponce DP, Maturana JL, Cabello P, Yefi R, Niechi I, Silva E, et al. **Phosphorylation of AKT/PKB by CK2 is necessary for the AKT□dependent up□regulation of β□catenin transcriptional activity.** Journal of cellular physiology. 2011;226(7):1953-9.

24. Wang G, Ahmad KA, Harris NH, Ahmed K. **Impact of protein kinase CK2 on inhibitor of apoptosis proteins in prostate cancer cells.** Molecular and cellular biochemistry. 2008;316(1-2):91-7.

25. Duncan JS, Litchfield DW. **Too much of a good thing: the role of protein kinase CK2 in tumorigenesis and prospects for therapeutic inhibition of CK2.** Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2008;1784(1):33-47.

26. Hessenauer A, Schneider CC, Götz C, Montenarh M. **CK2 inhibition induces apoptosis via the ER stress response.** Cellular signalling. 2011;23(1):145-51.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Sarah Sadiq	Sampling, Immunolist chemistry, article writing	
2	Dr. Abdul Khaliq Naveed	Supervision of research Guidance in article weiting	
3	Dr. Shahid Jamal	Histopathological scoring & Supervision in IHC	
4	Dr. Aiza Sadia	Histopathological Scroing & imaging	