International Journal of Medicine Research
www.medicinesjournal.com
ISSN: 2455-7404
Received: 31-12-2021, Accepted: 15-01-2022, Published: 31-01-2022
Volume 7, Issue 1, 2022, Page No. 3-8

Role of HSPA1A in hyperthermia induced stress response in cancers

Muneera Mohamed Sahib, Paolo Marsico
Department of Medical Science, Chester Medical School, University of Chester, Chester, United Kingdom

Abstract
Modulation in current Cancer treatment approaches is inevitable in order to minimise the undesirable aftereffects produced by currently available conventional treatments. Interestingly, the stress proteins elicit stress signals that call up the immune cells to act accordingly, resulting in rendering cancer survival and on the other hand, in certain situations, heat shock protein responses hold back the cells immune system, helping cancer progression. On that account, a clear-cut understanding of the role of these molecules within the cells and at the cell surface levels would be useful in considering appropriate treatment approaches in different cancers [1,2]. This study shows the effect of hyperthermic treatment and associated HSPA1A response in leukaemia cell lines U937 and colorectal cancer cell lines HT-29. The effects of hyperthermia exposure at 42 °C evaluated for cell viability by MTS and propidium iodide assays, apoptosis and necrosis were measured by FITC Annexin V and PI assay. The expression status of HSP A1A proteins upon hyperthermic treatment were also analysed in this study in both U937 and HT-29 cancer cell lines. Hyperthermia treatment for 1 hr showed cytotoxicity in both the cancer cell lines tested with increase in HSPA1A expression profile. Hence considering the side effect limitations and cytotoxity exhibited by hyperthermia makes it an effective cancer treatment approach.

Keywords: hyperthermia, apoptosis, HSPA1A, necrosis

Introduction
Cancer patients rely on conventionally available primary treatment strategies, which mainly comprise of radiation therapy and several chemo agents for prolonged survivals. Although, these are highly potent and efficient first line cancer treatment strategy, the side effects reflect high concern, and hence, treatments with lessening side effects and improved efficiency are needed [2]. Hsp proteins normally serve as a mechanism of self-defence against cellular oxidative stress, infection from biological entities or pathophysiological exposures [3]. As a result of cell death, heat shock proteins are released into the extracellular environment, where they successfully interact and connect with receptor proteins of the immune cells, to generate inflammatory signals as warning responses [4]. The considerable rise in HSP's in cancer cells are due to the cellular stress events experienced from undesirable transfiguration during cancer evasion [5]. Heat shock proteins are inevitable factors in protein folding, the unstable transfigured proteins resulted from mutation in cancer cells command for enhanced protein folding, which in turn generate intracellular stress leading to increase in HSP expression status in tumours. Several *in vivo* reports have revealed the requirement of HSP 72 for cancer survival and progression [6]. Clinical phases of pharmacological agents that effectively targets HSP 72 in cancers are under developmental stages. Several reports have drawn attention of heat shock proteins as eminent markers for cancer diagnosis due to their increased expressive status in certain cancer types. These proteins are classed according to their molecular sizes. Similarly inclined expression of both HSP 60 and HSP 70 are reported to promote cancer proliferation of multiple malignancies, which includes cancers of the prostate, intestinal and respiratory organs. [7]. High levels of HSP 110 proteins are associated with the progression of non- Hodgkin's lymphoma, skin and colon cancers [8]. These proteins tend to reverse the apoptotic process initiated by signalling pathways or through therapeutic agents.
Hence, considering the attention of HSP proteins in cancer treatments, hyperthermic approach of cancer destruction is an interesting treatment strategy, which exhibit significant cancer cell death, when exposed to temperature elevation of 42°C [9]. Heat exposure result in induction of heat shock proteins, which, in turn trigger cellular immune system to act simultaneously, resulting in targeting cancer cells generating HSP's [10]. Studies show the application of hyperthermic treatment using electronic systems along with radiation treatment and adjuvant chemotherapy for treating solid tumours [11]. Hyperthermic treatment have reported to show enhanced cell death at temperatures of 40-44°C in treating solid tumours, Application of hyperthermia blend with conventional cancer treatments evidence to signal elevation of heat shock proteins, resulting in improved treatment outcome *In vitro* and *in vivo* investigations in colorectal carcinoma [12]. This study investigated the effect of hyperthermia treatment *in vitro* and the expression profile of HSPA1A in leukaemia cell lines U937 and colorectal cancer cell lines HT-29.

Materials and methods

The human leukaemia cancer cell line U937 (85011440) and colorectal cancer cell line HT-29 (91072201) were purchased from the European culture collections. EMEM (Eagle's Minimal Essential Medium) and RPMI (Rosewell Park Memorial Institute 1640) was purchased from Lonza UK. MTS reagent (4,5-dimethylthiazol-2yl)-5-(3- carboxy methoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium from Promega, UK. PI reagent (Sigma Aldrich UK), FITC Annexin V (BD Pharmingen, UK). HSPA1A primary antibody was purified in laboratory at Chester Medical School, UK and HSPA1A secondary antibody (extravidin FITC) was purchased from Sigma Aldrich UK.

Cell culture, treatment and evaluation of cell viability

Cancer cell lines U937 was maintained in RPMI cell culture medium and HT-29 cell lines were maintained in EMEM medium at 37°C in incubator, maintaining standard culture conditions. The cells (3ml) were pooled into sterile 15 ml falcon tubes and exposed to heat treatments using a preheated water bath setup at 37°C and 42°C for 1h duration. Cells following subsequent treatments were placed in 37°C for recovery for 2h duration. Untreated U937 and HT-29 cells were used as reference. The cells were then transferred to microtiter plates and incubated at 37°C for 24h. Following incubation, cells were evaluated using MTS assay for cell viability or PI assay for cell necrosis. Cells were extracted and subjected to measurement of apoptosis and necrosis by annexin V and PI assay and expression of heat shock protein HSPA1A using BD Bioscience flow cytometer. All data were statistically evaluated using GraphPad prism 9 software.

MTS assay

Cancer cells lines following heat exposure treatment were subjected with addition of MTS reagent (20µl)/per well of the 96 well microtiter plate containing treated cells. Cell lines following treatments were measured for cell viability using plate reader at 490nm absorbance.

PI Assay

Cancer cells lines following heat exposures were evaluated for cell necrosis by PI assay using propidium iodide dye treatment in working concentration 1:200. The cells were then incubated at 18°C in the dark for 20 minutes. The cells were measured for necrosis using Varioskan LUX plate reader at 530/640 nm.

Evaluation of apoptosis/necrosis using Annexin V and PI assay

Cancer cells following hyperthermia treatments were transferred onto micro titre assay plates. The cells were centrifuged and resuspended in 100 µl cold PBS (phosphate buffered saline solution) for 3 consecutive intervals. The hyperthermia exposed cells were subjected to annexin V and PI reagents in 2.5µl volume each prediluted using BD Bioscience 1X binding buffer. Separate sets of cells treated with either annexin V or PI were placed as subsequent controls. The cells were placed in shaker and transferred in dark for 15 minutes and incorporated with 1X BD bioscience binding buffer and the plates were analysed using flow cytometer in 1 hr duration.

Evaluation of cell surface HSPA1A expression

Cancer cell lines U937 and HT-29 following hyperthermia treatment and incubation for 24 h ($1x10^6$ per ml) were pooled and centrifuged at 500 x g for 5 min and resuspended in appropriate culture mediums, transferred on to 96 well microtiter plates. The cells were then subjected to washing using cold DPBS solution for 3 consecutive intervals. The cells were centrifuged and added with blocking buffer (100µl) for 5 minutes and subsequently centrifuged and resuspended with primary HSPA1A antibodies [1:100 concentration; antibody: Foetal bovine serum blocking buffer-5%v/v in Dulbecco's phosphate buffered saline (DPBS)], the cells were refilled with FITC labelled secondary antibody (1:200) and incubated for duration of 10 min., 50 µl of blocking buffer were added to the cells and subjected to centrifugation. The cells were resuspended with DPBS (100µl) and evaluated for protein expression by flow cytometer.

Results

The cell viability measured by MTS assay with exposure at 37°C & 42°C following 24 h incubation period in U937cells and HT-29 cells showed decline in cell viability of 59.4 % & 27.8 % respectively (Fig 1A & 2A). No cell necrosis was observed in both cell lines analysed by PI assay (Fig 1B & 2B).

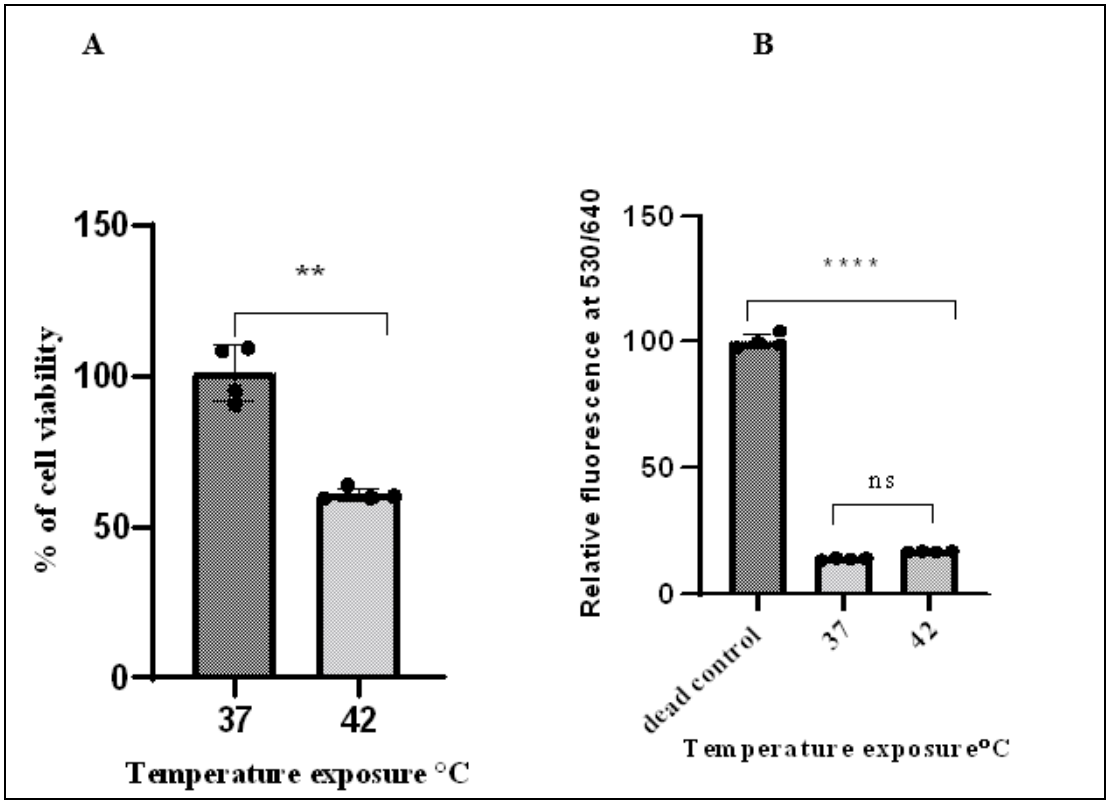

Fig 1: The U937 cancer cell lines treated with temperatures 37°C and 42°C for 1h duration followed with 2h recovery and incubation at 37°C for 24h duration. The cells were evaluated by (A) MTS assay. The data were statistically analysed by paired T-test ** (P 0.002) mean ± SD (n= 4) showed significant difference between control at 37°C and treated group at 42°C. (B) PI assay data analysed by one-way Anova test and Sidak's multiple comparison test showed insignificant difference between control at 37°C and treated group at 42°C mean ± SD (n= 4).

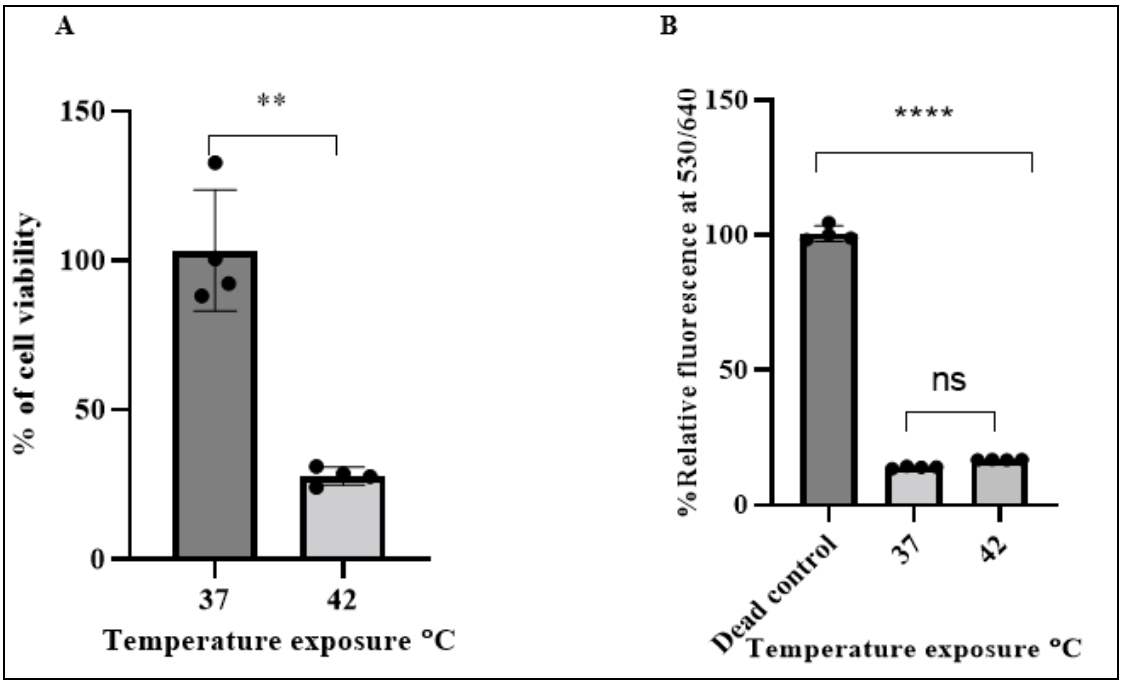

Fig 2: The HT-29 cancer cell lines exposed to 42°C for 1h duration followed with 2h recovery and incubation at 37°C for 24h duration was evaluated for cell viability. (A) MTS assay, the data were statistically analysed by paired T-test ** (P 0.0049) mean ± SD (n= 4) showed significant difference between control at 37°C and treated group at 42°C. (B) PI assay data analysed by one-way Anova test and Bartlett's multiple comparison test showed significant difference between dead control and cells at 37°C & 42°C ****(P<0.0001); insignificant difference between live cell control at 37°C and treated group at 42°C mean ± SD (n= 4).

Evaluation of heat shock exposure mediated response by flow cytometer

The flow cytometric evaluation presented a significant decline in live cells due to hyperthermic treatment in both U937cells and HT-29 in contrast to cell viability of control cells (91% and 92%), which declined to 66% and 54.4% respectively (Fig 3 & 4). The data also showed apoptosis (early & late apoptosis) of 28.1% in U937 and 34.4 % in HT-29 cells. The data finalize that the cell death resulted from hyperthermia was mostly by apoptosis as limited cells of U937 11% and HT-29 (11.2 %) cells underwent necrosis. The cell surface expression of HSPA1A proteins evaluated by flow cytometer exhibited an increase in this protein profile of 15% % in U937 and 39.4 % in HT-29 cancer cells in contrast to control cell lines maintained at 37°C, which showed HSPA1A expression of 4% in U937 and 18% in HT-29 respectively (Fig: 5 A & B).

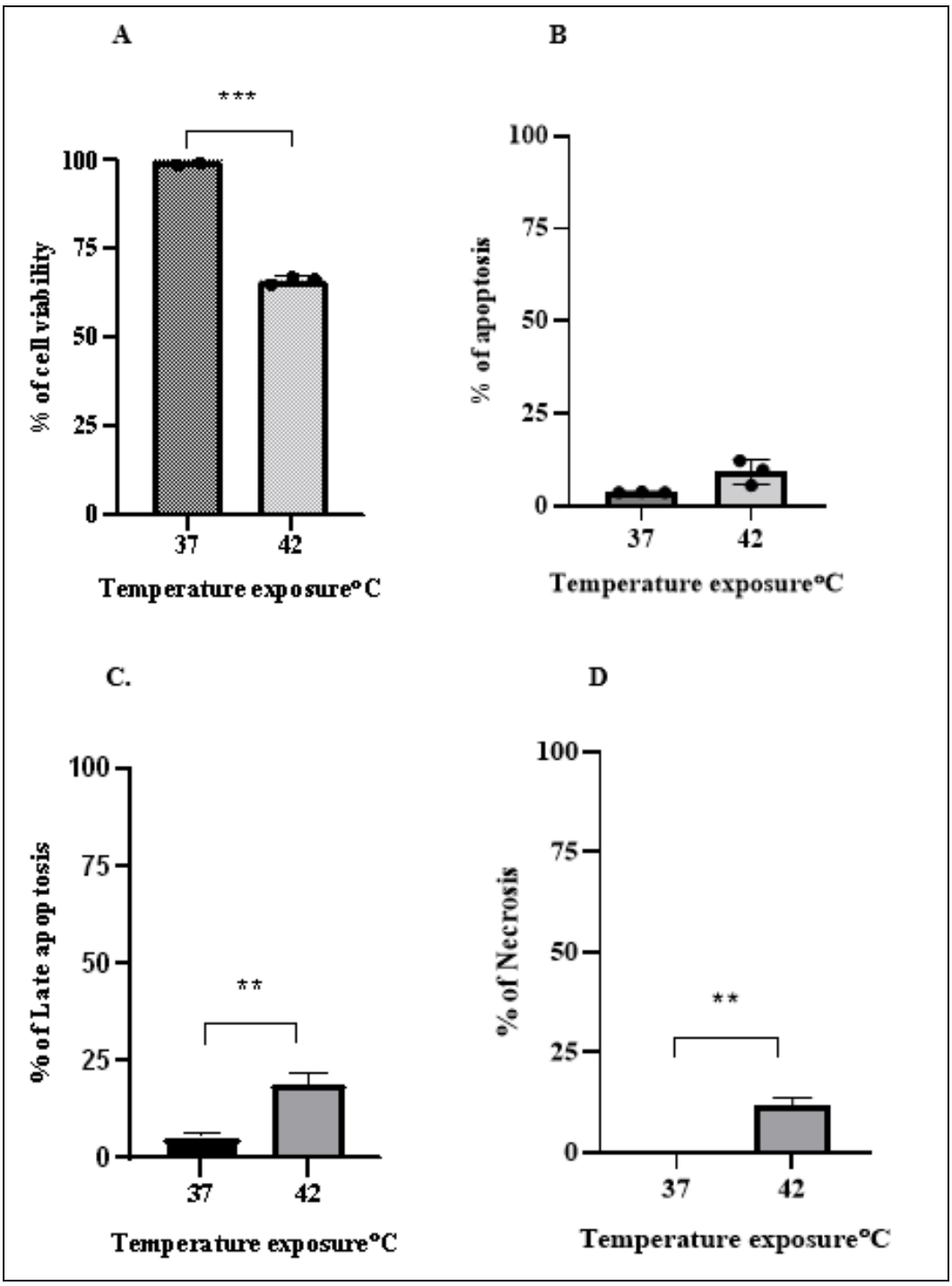

Fig 3: The U937 cancer cell lines exposed to 42°C for 1h duration followed with 2h recovery and incubation at 37°C for 24h duration was evaluated for (A) cell viability, (B) apoptosis, (C) late apoptosis & (D) necrosis by annexin V and PI assay. The data statistically analysed by paired T-test showed significant difference between control at 37°C and treated group at 42°C for % of cell viability ****(P 0.0003); % of late apoptosis **(P 0.0099), % of necrosis **(P 0.0063).

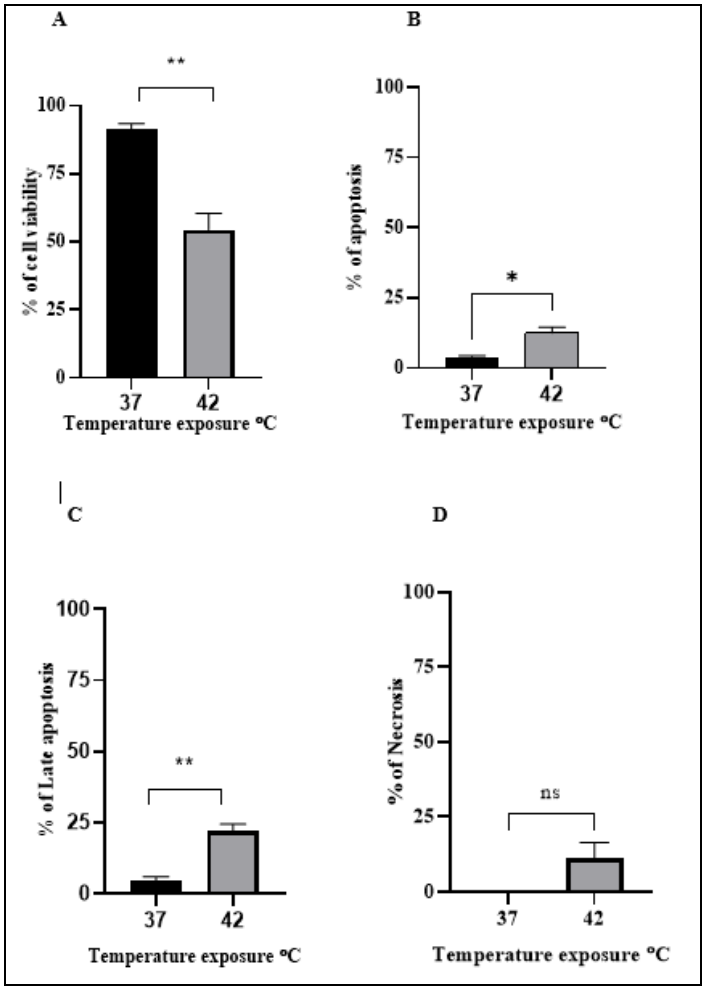

Fig 4: The HT-29 cancer cell lines were exposed to 42°C for 1h duration followed with 2h recovery and incubation at 37°C for 24h duration was evaluated for cell viability, apoptosis, late apoptosis, necrosis by annexin V and PI assay. The data statistically analysed by paired T- test showed significant difference between control at 37°C and treated group at 42°C for % of (A) cell viability **(P 0.0045); % of (B) apoptosis *(P 0.0187), % of (C) late apoptosis **(P 0.0017), % of (D) necrosis (P 0.0676) mean ± SD (n= 3).

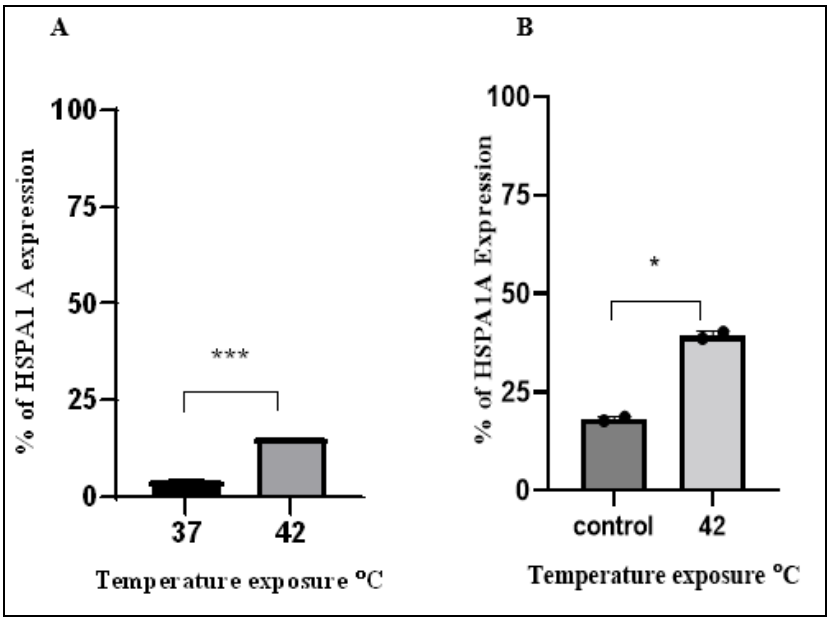

Fig 5: Expression of cell surface HSPA1A: The cancer cell lines were exposed to 42°C for 1h duration followed with 2h recovery and incubation at 37°C for 24h duration was evaluated for HSPA1A protein by flow cytometer in U937 and HT-29 cell lines. The data statistically analysed by paired T-test showed significant difference between control at 37°C and treated group at 42°C for in (A) U937 cell lines ***(P 0.0008); (B) in HT-29 cell lines *(P 0.0105).

Discussion and Conclusion

The HSP 70 proteins are studied to have an eminent role in amelioration of several cancers. Hyperthermia administration has reported apoptosis presented with enhanced expression of HSPA1A proteins in liver carcinomas [13]. Heat exposure at 42°C in this study resulted in significant rise in HAPA1A, which resulted in significant apoptotic death in both leukaemia and colorectal cancer cell lines investigated. Hyperthermia treatment is reported to be highly efficient in targeting in depth solid tumours. Heat exposure causes disruption in cancer cells, expelling impaired proteins towards extracellular locations, these proteins are automatically tracked by immune cells as warning signs, directing immune responses. Recent reports show the role of hyperthermia in lung cancers alongside β-elemene which resulted in promoting transcription of apoptotic molecules mainly BAX and p21, resulting in termination of cell cycle and hampering further progression of the tumour [14]. Besides accelerating the apoptotic process, hyperthermia combinations therapies halt the cancer cells counter protective mechanism of self-repairing and also help in overriding the resistance of cancer cells to currently available cancer treatments [15]. Hyperthermia induced cytotoxicity are studied to be accelerated by antioxidant activity, which in turn initiate the apoptotic cascade in leukemic cancers. Hyperthermia treatment in this present investigation in turn resulted in developing cellular stress, which was evidenced by enhanced expression status of HSPA1A in cancer cells, these proteins upon migrating to the exterior of the cell may call upon the immune system to act accordingly resulting in apoptosis. Hence, taking together the anticancer role of hyperthermia, its potential role in overriding resistance, when used in combination with conventional therapies and side effect limitations, makes hyperthermia as an exceptional treatment approach for cancers.

References

1. Daming Zou, Xiang Yang Wang, John Subjeck. Unfolding the role of large heat shock proteins. Frontiers In Immunology,2016:7:1664-3224.
2. G Multh off. Heat Shock Proteins in Immunity. Molecular Chaperones in Health and Disease. Handbook of Pharmacological Medicine,2006:172:279-304.
3. Suman Chatterjee, Timothy F Burns. Targeting Heat Shock Proteins in Cancer: A Promising Therapeutic Approach. International Journal of Molecular Sciences,2017:18(9):1978.
4. Calderwood SK, Gong J, Murshid A. Extracellular HSPs: The Complicated Roles of Extracellular HSPs in Immunity. Frontiers In Immunology,2016:7:159.
5. Paul Workman. Altered States: Selectively Drugging the Hsp90 Cancer Chaperone. Trends in Molecular Medicine,2004:10(2):47-51.
6. Gong J, Weng D, Eguchi T, Murshid A, Sherman MY, Song B *et al*. Targeting the Hsp70 Gene Delays Mammary Tumor Initiation and Inhibits Tumor Cell Metastasis. Oncogene,2015:34:5460-5471.
7. Hwang YJ, Lee SP, Choi Y H, Kim M J, Lee C H, Lee J Y, Kim D Y. Expression of heat shock protein 60 kDA is Upregulated in Cervical Cancer. Yonsei Medical Journal,2009:50(3):399-409.
8. Coralie Dorard, Aurelie de Thonel *et al*. Expression of a Mutant HSP110 Sensitizes Colorectal Cancer Cells to Chemotherapy and Improves Disease Prognosis. Nature Medicine,2011:17:1283-1289.
9. Anna Jolesch, Elmer K, H Bendz, R.D Issels, E. Noessner. HSP 70, a Messenger from Hyperthermia for the Immune System. European Journal of Cell Biology,2012:91(1):48-52.
10. Horowitz M, Robinson SDM. Heat Shock Proteins and the Heat Shock Response During Hyperthermia and its Modulation by Altered Physiological Conditions. Progress in Brain Research,2007:162:433-446.
11. Gadaleta Caldarola, G S Infusino, I. Galise, G. Ranieri, G. Vinciarelli, V. Fazio, R. Divella, A. Daniele, G. Filippelli, C. D. Gadaleta. Sorafenib and Locoregional Deep Electro Hyperthermia in Advanced Hepatocellular Carcinoma. A Phase II Study. Oncology Letters,2014:8(4):1783-1787.
12. Petra Schildkopf, Benjamin Frey, Oliver J Ott, Rubner, Multhoff G, Sauer R, Fietkau R *et al*. Radiation Combined with Hyperthermia Induces HSP 70-Dependent Maturation of Dendric Cells and Release of Pro-inflammatory Cytokines by Dendritic Cells and Macrophages. Radiotherapy and Oncology,2011:101(1):109-115.
13. Yang KL, Huang CC, Chi MS, Chiang HC, Wang YS, Hsia CC *et al*. Invitro Comparison of Conventional Hyperthermia and Modulated Electro-Hyperthermia. Oncotarget,2016:7(51):84082-84092.
14. Zhibing Wu, Ting Wang, Yanmei Zhang, Zhishuang Zheng, Shuhuan Yu, Saisai Jing, Sumei Chen, Hao Jiang, Shenglin Ma. Anticancer effects of β-Elemene with Hyperthermia in Lung Cancer Cells. Experimental and Therapeutic Medicine,2017:13(6):3153-3157.
15. Arlene L Oei, Lianne EM Vriend, Johannes Crezee, Nicolaas AP Franken, Przemek M Krawczyk. Effects of Hyperthermia on DNA repair Pathways: one treatment to Inhibit them all. Radiation Oncology,2015:10:165.