ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Correlated expression profiling of *dicer1* and *tlr* genes across bubaline tissues vis-à-vis in PBMCs challenged with tlr agonists

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Manuscript Info

Manuscript History:

Received: 19 August 2015
Final Accepted: 26 September 2015
Published Online: November 2015

Key words:

Gene-expression; qRTPCR; *dicer1*; *tlr*; miRNA; Buffalo

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Abstract

Dicer, a Ribonuclease III enzyme, plays key role in the biogenesis of microRNAs and small interfering RNAs and is essential for development, cell differentiation and immune regulation. On the other hand, Toll like receptors are non-catalytic, pattern recognition receptors, which play central role in innate immunity against wide range of pathogens. In the present study, differential expression of bubaline *dicer1* enzyme and Toll like receptors (*tlr*3 and *tlr*4) genes in nine different tissue samples and peripheral blood mononuclear cells (PBMCs) were profiled using real time PCR (TaqMan chemistry). All the genes exhibited highly differential pattern across the tissues studied, with the highest expression in heart for all the genes and low expression was observed in reproductive tissues (ovary and testis). Moreover, the tissue expression profile of *dicer1* enzyme has been reported for the first time in *Bubalus bubalis*. In order to find any correlation between the expression of *tlr*s and *dicer* in case of any bacterial or viral infection, their expression were studied in bubaline PBMCs challenged with *tlr*4- and *tlr*3- ligands viz. LPS (component of gram negative bacteria) and PolyI:C (synthetic analogue of dsRNA molecule), respectively; thus mimicking the infection *in vitro*. Upon ligand stimulation, *dicer1* gene expression was found to increase hand-in-hand with the increased expression of respective *tlr* genes. The study revealed positive association between the expressions of *dicer1* vis-à-vis *tlr*3 and 4 genes in bubaline PBMC, indicating that specific viral or gram negative bacterial infection co-regulates the expression of *dicer1* in PBMC. Thus, this finding warrants extensive study on identification of miRNAs associated with disease tolerance or susceptibility, which could be modulated by *tlr*-ligands through increased Dicer I activity in livestock.

INTRODUCTION

Dicer is the major enzyme involved in biogenesis pathway of both miRNAs and siRNAs and thus plays a central role in RNA interference. It has got functions in gene regulation and genome organization. Dicer has been implicated to play role in diverse processes such as follicle development (Luense et al., 2009), oocyte maturation (Liu et al., 2010), spermatogenesis (Yadav et al., 2013), embryonic development (Yang et al., 2005), chromatin remodeling (Fukagawa et al., 2004), stem cell maintenance (Forstemann et al., 2005), fragmenting chromosomal DNA during apoptosis (Nakagawa et al., 2010) and immune regulation (Cobb et al., 2006). Dysregulated *dicer1* expression has also been associated with carcinoma of different organs, namely, lung (Karube et al., 2005), breast (Grelier et al., 2009), cervix and endometrial adenocarcinoma (Zighhelboim et al., 2011) as well as prostrate (Bian et al., 2015) and ovarian cancer progression (Merritt et al., 2008). On the other hand, toll like receptors are important pathogen recognizing receptors (PRRs) involved in the recognition of microbial markers referred to as pathogen

associated molecular patterns (PAMPs) and plays critical role in the host-pathogen interaction via innate immunity. The repertoire of bubaline *tlr* consists of 10 genes (*tlr*1 to 10). *tlr*3 recognizes the viral nucleic acid i.e. double stranded RNA of viral origin as well as its synthetic analogue poly I:C (Alexopoulou et al., 2001) while *tlr*4 recognizes the bacterial pathogen, particularly Lipopolysaccharides (LPS), a component of gram negative bacteria and plays important role in linking innate and adaptive immunity (Akira et al., 2001). The expression of *dicer* has not been correlated with the *tlr* genes in buffalo. In the present study the expression profile of *tlr*3 and 4 has been associated with *dicer1* to determine the relationship of innate immune system and Dicer activity in bubaline PBMCs.

Dicer enzyme has been well characterized in humans but bubaline *dicer1* has not been studied. There are few qRT-PCR analyses of *tlr* expression in bovine and bubaline tissues (Vahanan et al., 2008; Vignesh et al., 2012). The present study has been designed to study the tissue specific expression profile of *dicer1* enzyme, *tlr*3 and *tlr*4 using real time PCR-TaqMan chemistry, in Indian water buffalo and a step towards exploring any relationship between expression of *tlr*s and *dicer* in normal, healthy tissues as well as in ligand inoculated bubaline PBMCs culture. The selected tissues are important from physiological point and were available in the slaughter-house from the slaughtered buffaloes.

MATERIALS AND METHODS

Sample collection

Tissue Samples: Nine different tissue samples (heart, liver, kidney, brain, ovary, testis, udder, horn base and hoof base) of adult buffaloes were collected from the slaughter house (M.K. Overseas Pvt. Ltd., Samgauli, Derabassi, Mohali, Punjab). The tissues were immediately stored in RNA later solution at -80°C until RNA extraction.

Blood Samples: 10 ml of peripheral blood was aseptically collected by jugular vein puncture method (according to IAEC guidelines) of adult, female Murrah buffaloes (n=4; for each subset of experiment viz. for tissue wise expression and culture stimulation experiments) maintained in the Dairy farm of the university. The PBMCs were immediately isolated separately from the freshly collected blood following density gradient centrifugation using Hisep1077 (Himedia) and stored at -80°C in RNA later solution until RNA extraction.

PBMCs culture and stimulation with *tlr* ligands

PBMCs (pooled) were cultured in 6-well culture plates containing RPMI-1640 growth medium (i.e. RPMI-1640 media supplemented with 10% serum and antibiotic- 50µg/ml Gentamicin, 100IU/ml of Penicillin, 100µg/ ml Streptomycin and 0.25 µg/ml Amphotericin) @ 2 X 10^6 cells per well in 2ml media, in CO_2 incubator at 37˚C. Initially, the cultured PBMCs in each well were challenged with *tlr*3 ligand (Poly I:C, synthetic analog of dsRNA, InvivoGen), and *tlr*4 ligand (LPS), separately in dose- and time- dependent manner, to find the dosage of ligands at which optimum/maximum expression of their respective *tlr* gene is observed.

- *tlr*3 ligand (Poly I:C) stimulation: Cultured PBMCs (isolated from blood of healthy animals; n=4 and pooled) were stimulated with two different doses of poly I:C (Final conc. 10µg/ml and 50µg/ml) for different time intervals : 1hr, 3hr, 6hr, 12hr, 18hr and 24 hr. Normal, untreated PBMCs just at start of incubation time (0 hr) and at end of each interval time was taken as control.
- *tlr*4 ligand (LPS) stimulation: The four different LPS doses (10ng/ml, 100ng/ml, 1000ng/ml and 2000ng/ml) were used to stimulate the PBMCs cultures (isolated from blood of healthy animals; n=4 and pooled). For each treatment group/dose of LPS, cultures were incubated for 2hr, 6hr, 12hr and 24hr, respectively. Non-stimulated normal PBMCs were also cultured for different time intervals, taken as respective control group.

Next, the experiment was performed with fresh PBMCs culture (isolated from blood of healthy animals; n=4 and pooled), now challenged with the ligand of optimum dose and incubation period. Co-regulated expression was studied between the *dicer1* and *tlr*4 gene (in LPS stimulated cultured PBMCs) vis-à-vis *dicer1* and *tlr*3 gene (in Poly I:C stimulated PBMC culture).

RNA extraction and cDNA synthesis

The tissue samples and pooled PBMCs stored at -80°C in RNA later solution were thawed at room temperature. Subsequently, the tissues were retrieved from RNA later Solution with sterile forceps and excess RNA later was quickly blotted away with an absorbent paper. The tissue samples (10mg each) were taken in 2ml micro-centrifuge tubes and were homogenized using bead-beater. PBMCs pellet were recovered by centrifugation and the RNA later solution as supernatant was discarded. The cells in RPMI-cultured PBMCs were also harvested by centrifugation @ 2500rpm for 10min. Total RNA was extracted from all the samples using Trizol (Ambion, Life Technologies, USA) method following manufacturer's instructions and the quality as well quantity of the extracted total RNA was assessed spectro-photometrically by Nanodrop (Thermo Fisher) and also by visualizing the ribosomal RNA bands via agarose gel electrophoresis. The total RNA samples that had O.D. 260/280 ratio between

1.8 and 2.0; and were showing clear ribosomal bands (28S and 18S) in agarose gel were selected for further processing. Total RNA (1µg) was reversed transcribed into cDNA using a Revertaid cDNA synthesis kit (Thermo Scientific) according to the manufacturer's instruction.

Real time PCR

Quantitative PCR was performed using custom designed (i.e. assay-by-design) and synthesized TaqMan assay (Applied Biosystems, Life technologies). The NCBI accession numbers of the sequences, specific to *Bubalus bubalis* species, that were used for custom designing of these assay were: DQ508811 (*tlr*3), HQ343416 (*tlr*-4), AB969677 (*dicer*) and DQ6611647 (Beta actin). The full length bubaline dicer sequence cds has been cloned (as partial overlaps), analyzed and submitted to DDBJ from our lab (Singh et al., 2015). The detailed sequences of primers and probes have been mentioned in Table 1.The real-time PCR reaction was carried out in duplicate/triplicate technical replicates in 20µl final volume containing 1X TaqMan master mix (TaqMan universal master-mix II with UNG, Applied Biosystems Life technologies, USA), primer-probe mix (900nM of each primer and 200nM of probe) and template cDNA (2µl). ß-actin was used as endogenous control. PCR overlay for real time amplification was: uracil inactivation phase (50°C for2 min), initial denaturation (95°C for 10 min) followed by40 cycles of denaturation (95°C for 15 sec), annealing +extension (60°C for 1 min). Threshold Cycle (Ct) values were calculated using the SDS software v.2.3 (Applied Biosystems, Life technologies, USA) with automatic baseline settings at threshold of 0.2.

Table 1: Detail of the sequences (5'to 3') of the Assay by Design (ABD) for the probe-primer mix for TaqMan Assay of Dicer1 enzyme, TLR3, TLR4 and Beta actin (ACTB, endogenous control)

Primer Name	Forward Primer (5'-3').	Reverse Primer(5'-3').	Reporter Sequence (Dye-FAM)(5'-3')
ACTB	cacggtgcccatctacga	gccagccaggtccagac	cccatgccatcctgc
Dicer1	gctgacgtgtacactgatcttacc	actccgcaaacgtttcatactca	cccctgagtaaattt
TLR3	acctcggccttaatgagattgg	gctctaggtatttgttgtaggaaaggt	cctggcctgtgagttc
TLR4	tgagcttcaatgatgtcattaccttagg	gaagatccaggtgttctagttgct	aagcccatgaagtttg

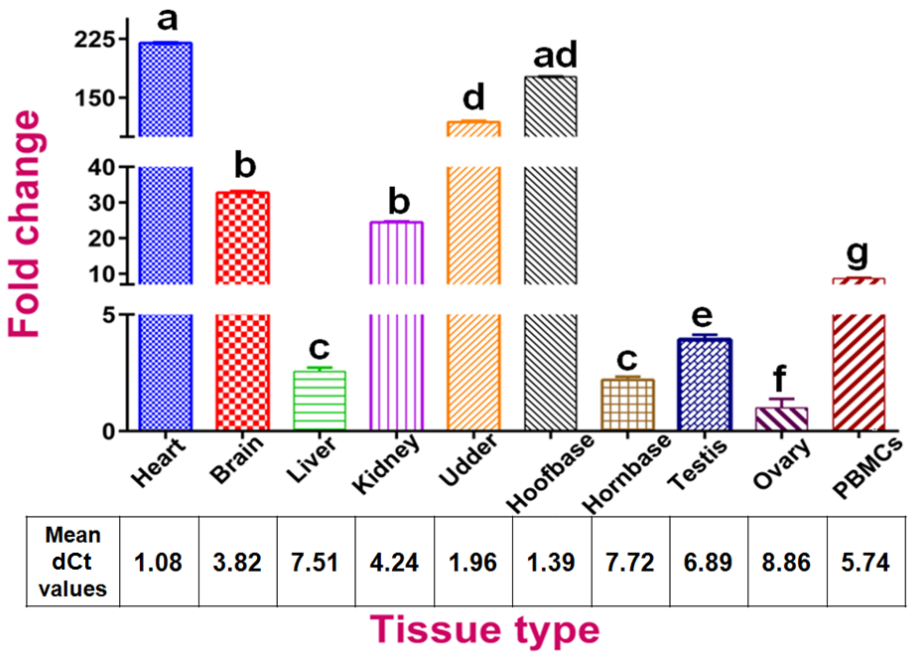
Analysis of QPCR Data: The QPCR data was analyzed for fold change using ΔΔCt method (Livak and Schmittgen, 2001). The dCt values (Ct values normalized to the endogenous control Ct) were subjected to ANOVA followed by post hoc test (Fisher's least significant difference), to determine any significant differences ($P<0.05$) among the levels of effects like, tissue of origin as well as doses and incubation period following *tlr*-ligand inoculation. The co-expression of *tlr*s and *dicer1* among tissues as well as in ligand treated PBMCs were determined using Pearsonian correlation coefficient (with Bonferroni probability of significance). The analyses were done using Systat version 13.00.05 (SSCP.Inc). The graphs have been generated using evaluation version of GraphPad Prism (ver. 6.05 for Windows), GraphPad Software, La Jolla California USA (www.graphpad.com).

RESULTS AND DISCUSSION

Tissue specific expression profiles of dicer1, tlr3 and tlr4: Quantifying the expression of protein or enzyme across the different tissues is an important initial step to investigate its functions as well as to provide a reference for comparing the expression in different physiological conditions.

A stable and regulated expression level of the dicer is expected to maintain the homeostasis of the miRNA production. Tissue specific relative expression of bubaline *dicer1*, (normalized against bubaline Beta actin), was determined after calibrating the expression with respect to that of ovary (Fig 1, 2 and 3, respectively), as the delta Ct value for the ovary tissue was highest. The experiment was performed twice, independently (each with technical replicates=2) and the expression levels presented for each tissue are the mean average taken from these two experiments. *dicer1* enzyme exhibited highly differential pattern of expression across the tissue being studied (Fig 1) with highest expression in heart followed by hoof base tissue. Although the expression was not significantly different between these two samples but it was significantly different from rest of the tissues at $P < 0.05$). Higher expression of *dicer1* was also observed in udder tissue, the expression not significantly different from hoof-base but significantly different from rest of the tissues at $P < 0.05$). The moderate and significantly similar expression levels were observed among brain and kidney; while in liver and horn-base, poor expression was detected. The lowest expression of *dicer1* was observed in ovary.

Fig 1: Relative expression profiling of *dicer*1 enzyme in terms of fold change with respect to ovary, in ten different normal tissues of the Indian water buffalo (*Bubalus bubalis*). No common symbol (a, b, c, d etc. symbols) between any two tissues indicate significant difference among the fold change (P<0.05).



Although Dicer enzyme has been well characterized in humans, mostly associated with various cancers but in livestock species (cattle and buffaloes) *dicer1* has not been studied. Expression of porcine *dicer1* mRNA has been detected in oocytes and in IVO produced blastocyst embryos (Stowe et al., 2012). Tissue distribution of two goose Dicer splice variants (gDicer-a, gDicer-b) and caspase-3 were detected by Hu and colleagues (2014) in six tissues by qRT-PCR. The mRNA levels of gDicer-a were found to be significantly higher (P< 0.05) in the pituitary than in other tissues with the exception of the oviduct, followed by the hypothalamus, and relatively lower levels in the liver, adrenal glands, and ovary. gDicer-b mRNA was expressed in all tissues at a constant level with no significant difference between each other (P< 0.05), but elevated levels were seen in the adrenal glands and hypothalamus. While other reports of characterizing the *dicer* expression are available in lower species. For example, tissue-specific expression profile of *dicer1* using real time PCR in 8 rainbow trout revealed abundance of *dicer1* in brain and liver. Expression profiling during different stages of embryonic development revealed maximum expression during embryonic genome activation (EGA) (Ramachandra et al., 2008). Luo and colleagues (2010) analyzed and revealed stage-specific expression profiles of Dicer and AGO1 by relative qRT-PCR in different developmental stages of parasite, with highest expression levels in the miracidium stage.

Expression of *tlr*s is important for the host response against pathogen. Relative expression of bubaline *tlr3* and *tlr4* across the tissues (technical replicates=2, for each tissue), was determined (normalized against bubaline Beta actin) after calibrating the expression with respect to that of ovary (Figs 2 and 3, respectively); as expression in ovary was low and also to compare the expression pattern for all the 3 genes under study. Maximum expression of *tlr*3 was observed in heart followed by brain, and udder with no significant difference from each other but significantly different (P <0.05) from rest of the tissues (Fig 2). In case of *tlr*4, expression was again found to highest in heart followed by udder and brain. Reproductive tissues (testis and ovary) showed lower expression of both *tlr*3 and *tlr*4, while least expression was observed in horn-base tissue. The expression levels of *tlr*4 between reproductive tissue (testis and ovary) and between kidney and PBMCs were found to be not significantly different (P < 0.05), whereas significantly different (P < 0.05) expression levels were observed across rest of the tissues (Fig 3).

Dhara and coworkers (2007) characterized the *tlr*3 in few tissues using real time PCR in water buffalo (*Bubalus bubalis*) and nilgai (*Boselaphus tragocamelus*) and found maximum expression, in testes, lung, kidney and skin. A very little expression was detected in brain, trachea, PBMNCs and dendritic cells. Vahanan and colleagues (2008) detected the presence of toll-like receptors (*tlr*s) 1–10 in few tissues buffalo including peripheral blood mononuclear cells and neutrophils, using reverse transcriptase polymerase chain reaction (RT-PCR) with bovine *tlr*-specific primers. However, the expression levels were not measured quantitatively. *tlr*3 was expressed in MNCs, spleen, lung, liver, heart and ovary. *tlr*3 was not expressed in neutrophils. Expression of buffalo *tlr*4 was found in all

cells and tissues tested expect kidney and uterus. The digital gene expression measured in the terms of RPKM values using RNA-Seq in 9 different tissues of buffalo revealed high expression of *tlr*4 in lungs and mammary glands; while *tlr*3 the expression was highest in hypothalamus and spleen; both the *tlr*s exhibited low expression in liver (Banerjee et al., 2012).

Fig 2: Relative expression profiling of *tlr*3 gene in terms of fold change with respect to ovary, in ten different normal tissues of the Indian water buffalo (*Bubalus bubalis*). No common symbol (a, b, c, d etc. symbols) between any two tissues indicate significant difference among the fold change (P<0.05).

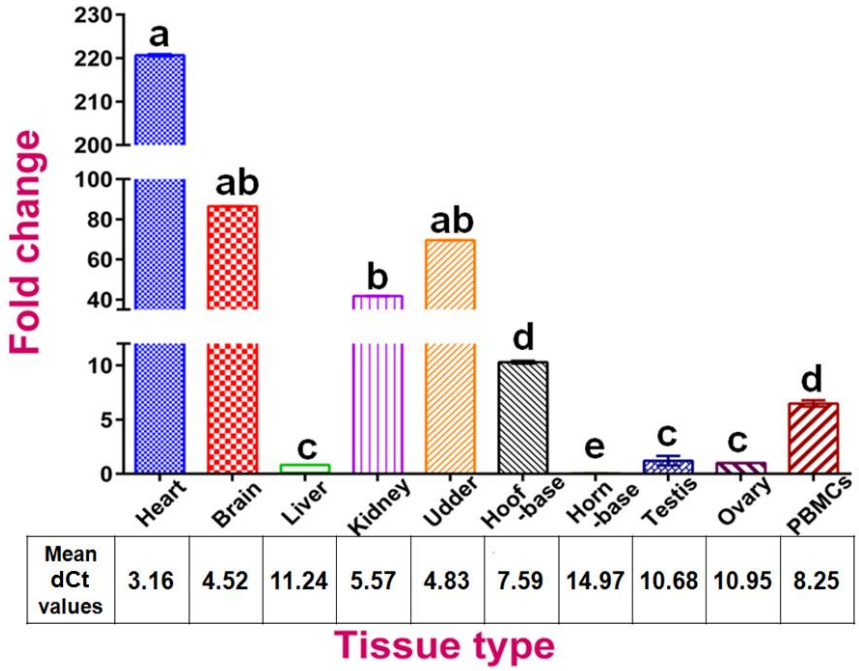
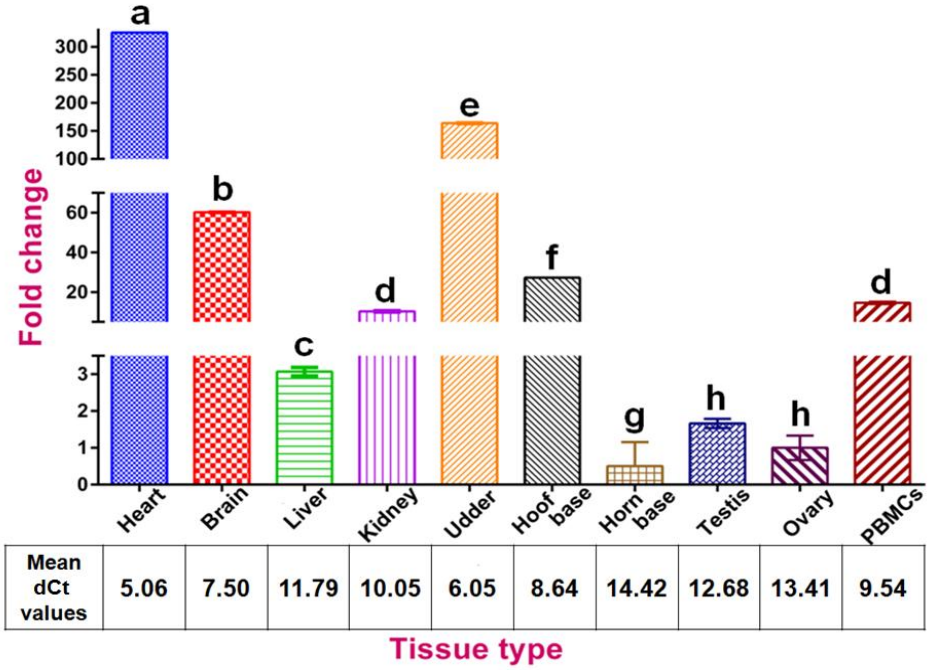


Fig 3: Relative expression profiling of *tlr*4 gene in terms of fold change with respect to ovary, in ten different normal tissues of the Indian water buffalo (*Bubalus bubalis*). No common symbol (a, b, c, d etc. symbols) between any two tissues indicate significant difference among the fold change (P<0.05).



More literature is available characterizing the *tlr* genes in cattle and pigs. Menzies and Ingham (2006) also identified and confirmed the expression levels of a these Toll-like receptors (*tlr*s 1–10) in selected bovine and ovine tissues using qPCR assays. Among the ovine tissues, peyer's patch, jejunum and mesenteric lymph nodes expressed all the 10 *tlr*s. While in bovine skin all *tlr*s apart from *tlr*6 were detected. *tlr*3 was the most abundant in the ovine jejunum whereas *tlr*4 was expressed at the lower limit of detection in all the tested tissues. Recently, Cheng et al., (2015) detected the expression of *tlr* genes (*tlr* 1 to 9) and two Anti-Microbial Peptide (AMP)-encoding genes (PBD-1 and PR-39) in the immune organs and tissues (thymus, spleen, blood, palatine tonsils, and mesenteric and pulmonary lymph nodes) of the two breeds pig breeds (Tibetan and Yorkshire pigs) at ages of 6, 12 and 24 weeks and suggested that Tibetan pigs have stronger innate immunity than those of Yorkshires with significantly higher transcript levels of the major *tlr* genes in most tissues of the immune system. Expression pattern of *tlr* genes (*tlr*1-10) in pig reproductive organs or tissues (ovary, oviduct, testis, and epididymis) have been reported by Marantidis et al., (2015) and similar to our results, the expression of *tlr* genes did not differ significantly in the analyzed reproductive tissues/organs.

The simple correlation coefficients between the dCt values of the target genes (*dicer1, tlr3 and tlr4*) over the range of tissues have been shown in the Table 2. The expressions of the genes under study are highly and significantly correlated in the healthy tissues.

Table 2: Pearson correlation matrix indicating the correlation coefficients between the genes (Dicer1, TLR3 and TLR4) in the healthy tissues of Indian buffalo

	Dicer	**TLR3**	**TLR4**
Dicer	1.000		
TLR3	0.836**	1.000	
TLR4	0.913**	0.934**	1.000

** P<0.01

***Co-regulated Expression of tlr3 and Dicer I*:** Expression profile of *tlr*3 gene at different time intervals (0hr, 1hr, 3hrs, 6hr, 12hr, 18hr and 24hr; technical replicates=2 each) in Poly I:C inoculated (50µg/ml) and non-inoculated (control) PBMCs culture (obtained from blood of healthy animals; n=4 and pooled) is presented as fold change relative to the expression in control at 1hr, which have maximum deltaCt value (Fig 4). Highest expression of *tlr*3 was observed at 12hr interval of ligand stimulation (i.e. ~12 folds expression than control at 1hr), though its expression was not significantly different from the expression in control PBMCs at 12 hr (i.e. ~10 folds expression than control at 1hr). Relative expression of *dicer1* along with the *tlr*3 expression was measured again at this dose-time combination in fresh PBMC culture, isolated from four healthy buffaloes separately and pooled (with technical replicates=3) (Fig 5). Normal expression of *dicer1* and *tlr*3 were comparable and upon ligand stimulation also, a significant ($P < 0.05$) and comparable increase in the expression levels of both *dicer1* (9.8 folds than control at 12hrs) and *tlr*3 (10 folds than control at 12hrs) was observed. No significant change in the expression levels of *tlr*3 gene was observed between the control and the Poly I:C inoculated PBMCs for different time intervals at the dose of 10 µg/ml (data not shown).

The correlation coefficients between the dCt values of the target genes (*tlr*3 and Dicer) for the Poly I:C stimulated (50µg/ml) and control (non-stimulated) PBMCs cultures have been shown in the Table 3. The expressions of the genes under study are highly and significantly ($P < 0.01$) correlated in the Poly I:C stimulated cultures.

Table 3: Pearson correlation coefficients (r) between the genes Dicer1 and TLR3 (on Poly I:C stimulation (50µg/ml for 12hr)) and genes Dicer1 and TLR4 (on LPS stimulation (100ng/ml for 6hr)) of PBMCs culture of Indian buffalo

S.No.	**Gene1**	**Gene2**	**Simple Correlation Coefficient**
1	Dicer	TLR3	0.695*
2	Dicer	TLR4	0.842**

* P<0.05
** P<0.01

Fig 4: Expression profile of *tlr*3 gene at different time intervals (0hr, 1hr, 3hrs, 6hr, 12hr, 18hr & 24hr) in Poly I:C inoculated (50µg/ml) and non-inoculated (control) PBMCs of the Indian water buffalo (*Bubalus bubalis*). No common symbol (a, b, c, d etc. symbols) between any two indicate significant difference among the fold change (P<0.05).

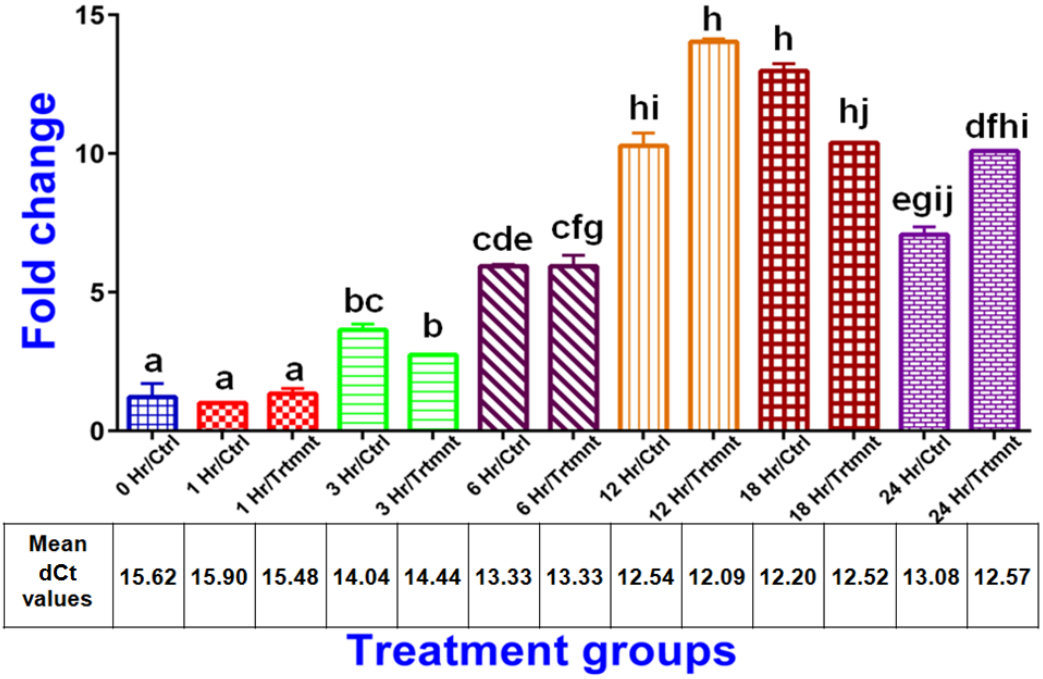
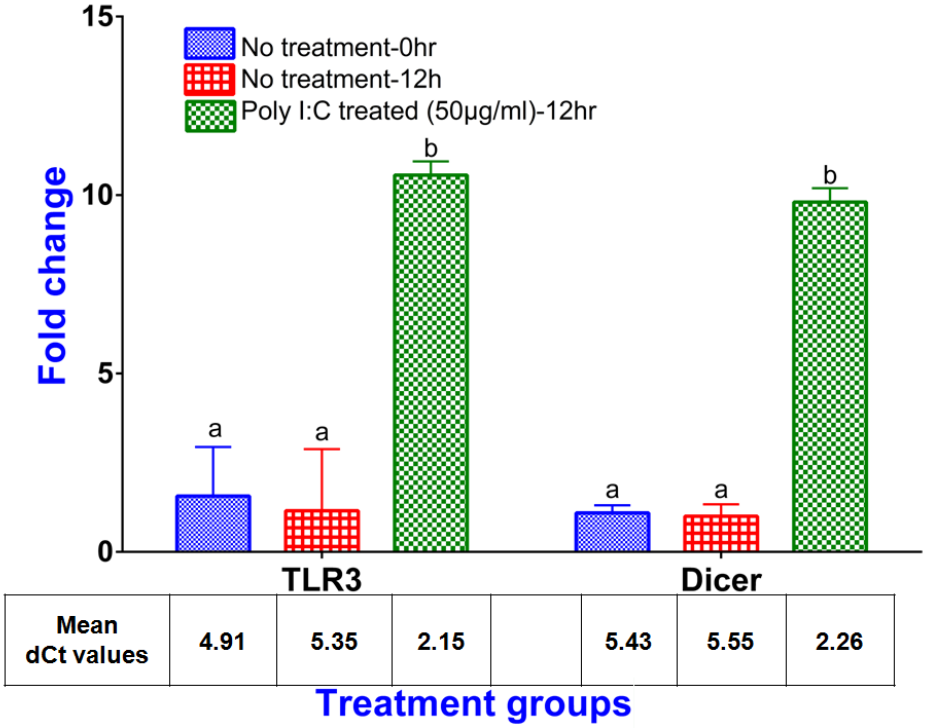


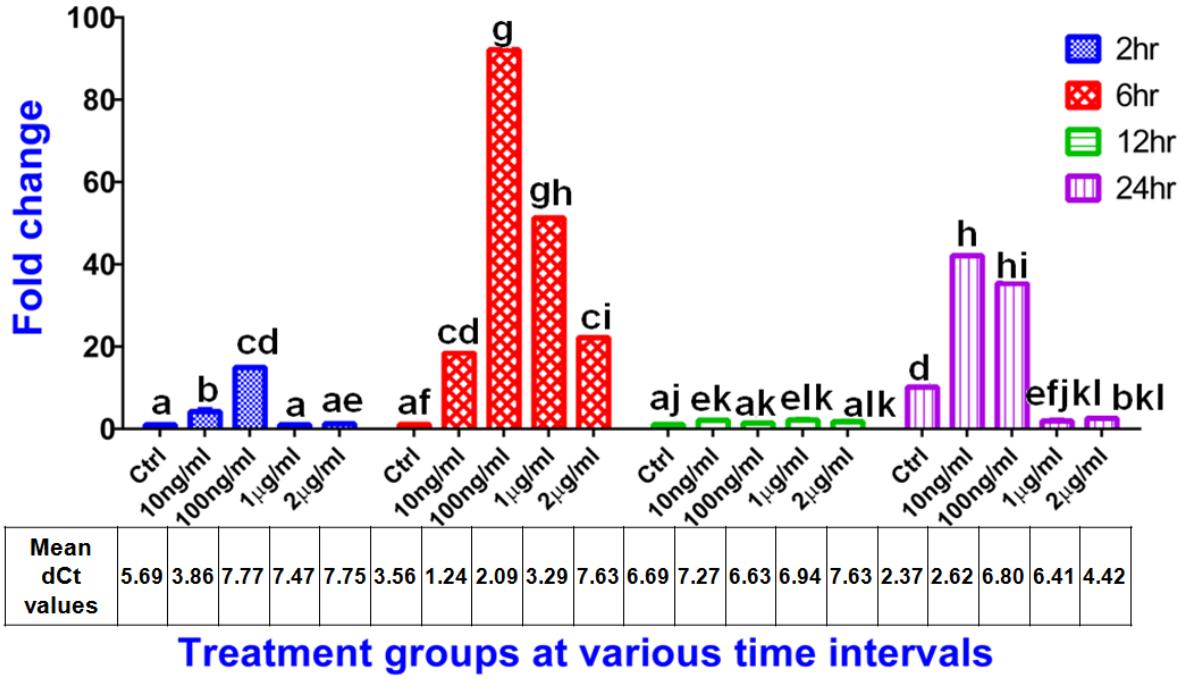
Fig 5: Expression of *tlr*3 gene and *dicer*1 enzyme at 12hrs in Poly I:C inoculated (50µg/ml) and non-inoculated (control) PBMCs of the Indian water buffalo (*Bubalus bubalis*). No common superscript (a, b, c, d etc. symbols) between any two indicate significant difference among the fold change (P<0.05).



***Co-regulated Expression of tlr4 and Dicer I*:** Relative expression profiling of *tlr*4 gene studied at different time intervals and with different doses of LPS stimulation (2 technical replicates each) in bubaline PBMCs culture (pooled, isolated from blood of 4 healthy animals) taking expression at 1000ng/2h as 1, being the least expression (Fig 6). At 2hr, 6hr and 24 hr, the expression of *tlr* increased with increasing dose from 10ng/ml to 100ng/ml as compared to control, but expression declined at higher dose at these time intervals. Maximum expression of *tlr*4 was observed at 6hr on LPS stimulation of dose @ 100ng/ml. Fresh PBMCs (pooled, isolated from blood of 4 healthy animals) were cultured and were stimulated with this particular dose and incubation time. Expression of *dicer1* was measured along with the *tlr*4 expression (technical replicates=3 each) in stimulated PBMCs at 0hr and 6hr compared with non-stimulated PBMCs at 0hr and 6hr. Relative expression was studied by taking expression of non-stimulated PBMCs at 6hr taken as 1 (Fig 7). *dicer1* exhibited significantly higher expression than *tlr*4 in normal (non-treated) as well as in LPS treated PBMCs. On LPS stimulation, *dicer1* expression increased significantly ($P < 0.05$) along with the significant ($P < 0.05$) increase in the expression of *tlr*4. But change in the expression level was very high in case of *tlr*4 (~73 times fold change than non-stimulated PBMCs at 6hr) as compared to change in the dicer expression (~7 fold change than non-stimulated PBMCs at 6hr).

A positive correlation was observed between the expression of *dicer1* and *tlr*4 genes in PBMCs on LPS stimulation. The correlation coefficients between the dCt values of the *tlr*4 and Dicer genes for the LPS stimulated PBMCs culture have been shown in the Table 3. The expressions of the genes under study are significantly ($P < 0.05$) correlated in LPS treated and control PBMCs cultures.

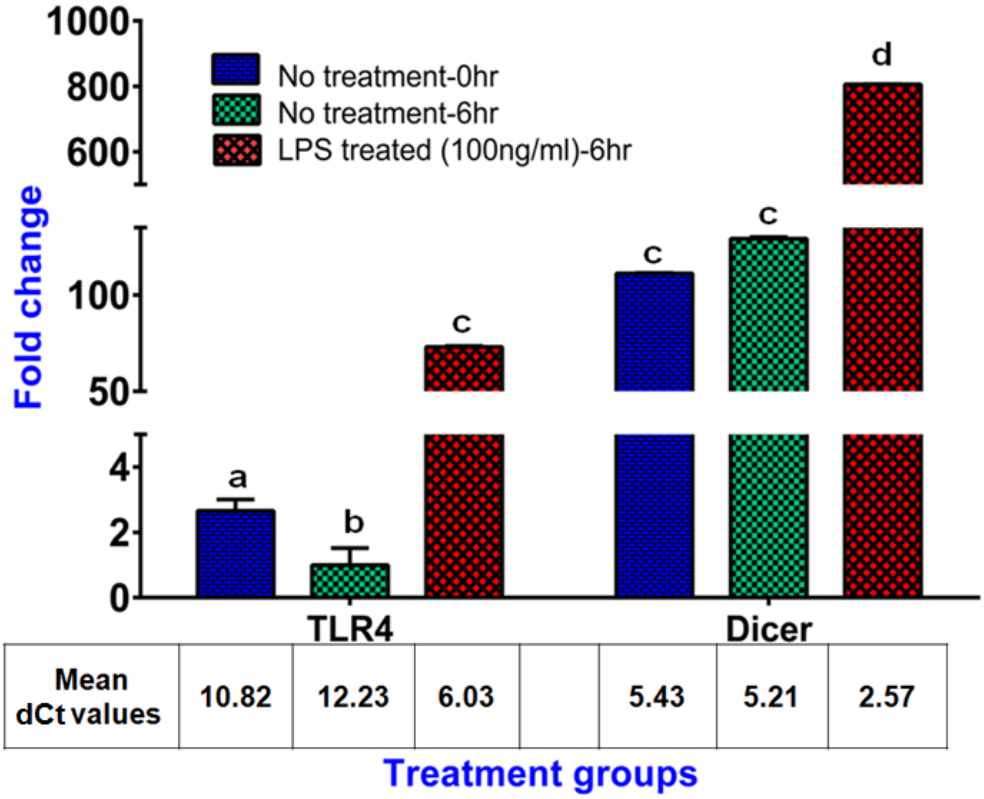
Fig 6: Expression profiling of *tlr*4 gene at different time intervals (2hr, 6hr, 12hr & 24hr) in LPS inoculated (Dose-10ng/ml, 100ng/ml, 1µg/ml & 2 µg/ml) and non-inoculated (control) PBMCs of the Indian water buffalo (*Bubalus bubalis*). No common symbol (a, b, c, d etc. symbols) between any two indicate significant difference among the fold change ($P<0.05$).



Innate response to LPS in bovine and human PBMCs and other specialized cells such as macrophages have been studied by many researchers and shown that LPS induced the expression of early response genes through binding with *tlr*4 (Janský et al., 2003, Palsson-McDermott et al., 2004). Doherty et al., 2013 studied the epigenetic regulation of induced immune response of LPS by stimulation the bovine PBMCs for 4hr with dose of 1µg/ml. Similarly, Poly IC triggered immune response is also well characterized (Huang et al., 2006). Erdinest et al., 2014 stimulated the HCE and HCF cells with Poly I:C at a dose of 25 µg/ml for *tlr*3 activation and maximal induction of TNF-α and IL-1β on 12 hours incubation. Dicer, a key RNAi component, has been implicated to play role in fragmenting chromosomal DNA during apoptosis (Nakagawa et al.,, 2010) and immune regulation (Cobb et al.,

2006). Dysregulated *dicer1* expression has also been associated with carcinoma of different organs. (Grelier et al., 2009, Zighelboim et al., 2011). Matskevich and Moelling (2007) demonstrated that knockdown of Dicer, led to a modest increase of virus production and accelerated apoptosis of influenza A virus-infected cells and thus Dicer is important for protection against influenza A virus infection. Despite of so many studies on characterization of Dicer and *tlr* genes, no reports are yet available ascertaining the correlation between the expression of *dicer1* and *tlr* genes. The present study has provided the evidence that Dicer expression increased in a correlated manner with the increased expression of *tlr* genes on LPS and PolyI:C stimulation of bubaline PBMCs

Fig 7: Expression of *tlr*4 gene and *dicer*1 enzyme in LPS inoculated (100ng/ml) and non-inoculated (control) PBMCs at 6 hours interval of the Indian water buffalo (*Bubalus bubalis*). No common symbol (a, b, c, d etc. symbols) between any two indicate significant difference among the fold change (P<0.05).



CONCLUSION

In the present study, varying degree of expression was observed across the tissues studied, with maximum expression in the heart tissue for all the genes studied i.e. *dicer1*, *tlr*3 and *tlr*4. However, these three genes exhibited lower abundance in the liver and reproductive tissues. Significant correlation among the expression level of the target genes was detected over the range of healthy tissues. Upon stimulation of cultured PBMCs with *tlr*-3 vis-à-vis -4 ligands, respectively, co-regulated pattern of expression was detected for *dicer1* gene. It may be hypothesized that in cases of bacterial and viral infections, there is increased co-regulated expression of *tlr* genes and *dicer1*, involved in the innate response. Further studies aimed at profiling the expression of miRNAs in PBMCs challenged with individual *tlr*-ligand, will demonstrate whether increase in Dicer expression has direct impact on up or down regulation of miRNA genes.

ACKNOWLEDGEMENT

The authors thankfully acknowledge the Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India (Project No. SR/FT/LS-22/2011), for providing the funds to conduct the research work.

STATEMENT OF ANIMAL RIGHTS

The study involved collection of tissue samples from the slaughter house as well as blood collection from healthy buffaloes. The work was approved by the Institutional Animal Ethics Committee (IAEC), GADVASU and all the ethical standards were maintained during the handling of the animals

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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