Research Article

Gene Profile Expression Related to Type I Interferons in HT-29 Cells Exposed to *Cryptosporidium parvum*

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Abstract

Background: *Cryptosporidium parvum* may contribute to upregulation or downregulation of host cellular genes among which type I (α, β) and type II (γ) interferons play key roles to eliminate infectious agents.

Objectives: The current study aimed at evaluating the expressed genes related to human type I interferon response in HT-29 cell line after exposure to *C. parvum* for six and 24 hours.

Methods: Subsequently, the overexpression and under expression of 84 human genes related to type I interferons were investigated using RT2Profiler™ PCR (polymerase chain reaction) Array.

Results: Four top overexpressed genes including *IL10, SH2D1A, MX1*, and *HLA-A* after six hours exposure, and 10 top overexpressed genes as *IL15, DDX58, CXCL10, NMI, MYD88, STAT3, IFNAR2, IFIH1, CASP1*, and *TLR3* were observed after 24 hours exposure. Five underexpressed genes such as *TIMP1, TYK2, IRF2, PML* and *IRF5* were monitored for six hours.

Conclusions: The current study findings revealed that the overexpressed genes *IL15, TIMP1*, and *SH2D1A* may have an important role to inhibit the invasion of *C. parvum*. Also, the overexpressed genes, namely *SH2D1A, MX1*, and *NMI*, may have antiviral properties while *TIMP1* may have anticancer properties. Further, the pertinent results demonstrated that the type I interferons and the relevant genes had significant effects on stimulating innate immune system against *C. parvum*.

Keywords: HT29 Cells, Type I Interferon Response, Gene Profile Expression, *Cryptosporidium parvum*, Iowa Strain

1. Background

Cryptosporidium is a coccidian protozoan parasite, which belongs to the phylum *Apicomplexa* and causes cryptosporidiosis. The spread of infection hinges on several factors such as environmental situation, host growing old, immune system status, geographic locality, and contact with infected humans and/or animals (1). Epithelial cells contribute primary and quick defense in opposition to cryptosporidiosis, and also activate immune effector cells to the infectivity position. The attachment of *Cryptosporidium parvum* to apical cell surface can stimulate host cell signal pathways and hence modify cell function (2). The development of *C. parvum* infection in intestinal epithelial cells is effectively controlled by both type I and II interferons (IFNs) (3). Type I IFNs bring cell-inherent antimicrobial states in infected and adjacent cells that confine the increase of infectious agents, mainly viral pathogens.

The investigation on type I IFNs in immunity to parasitic protozoa is limited; however, the role of type I IFNs showed that they reduce the severity of INF of *Leishmania major*, and that they can act in a straight line on host cells to induce parasite inactivation. Some studies showed that the development of *C. parvum* infection in intestinal epithelial cells is effectively controlled both by type I and type II IFNs. The enterocytes can produce type I IFNs in rejoinder to *C. parvum* infection and lead to the inhibition of parasite development (3). The cultivation of *C. parvum* in tissue culture provided a new approach to recognize different gene expressions and immunological responses to different parasite proteins. Initially, primary epithelial cell

culture (PECs) can stimulate different cellular genes after being exposed to *C. parvum* ([4]). Additionally, the continuous cell lines HCT8 and HT-29 showed good support for *C. parvum* ([5]). The implementation of advanced molecular tool can assess the gene expressions and their relative functions. The application of RT2Profiler™ PCR Array is a golden standard, which can measure low levels of gene expression ([6]).

2. Objectives

Since very few publications with focus on gene expression profile in the cells exposed to *C. parvum* oocysts can be found, the current study aimed at evaluating overexpressed and underexpressed genes related to type I INFs in HT29 cells infected by *C. parvum* using RT2 Profiler™ PCR Array.

3. Methods

3.1. Ethics Statement

The current study was granted by research deputy (Grant no: 94147) and ethically approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences.

3.2. Parasite Provision and Infection of Epithelial Cells

Cryptosporidium parvum oocysts (Iowa strain, BTF Company from Sydney –Australia) were kept in a solution of 0.01% Tween 20, 100 U/mL penicillin, and 100 mg/mL gentamicin, and stored at 4°C. To determine the infectivity of *C. parvum*, two-day-old neonatal rats (Razi Vaccine and Serum Research Institute, Karaj, Iran) were inoculated orally with 100,000 - 120,000 *C. parvum* oocysts ([7]). The shedding of the oocysts in the feces of neonatal rats were observed after eight days following the infection and confirmed with Ziehl–Neelsen staining.

3.3. Treatment of Oocysts

Oocysts were washed with 0.15 M/L phosphate-buffered saline (PBS; pH 7.2), centrifuged (3000 g for 15 minutes at 4°C) and treated with acidic H_2O (pH 2.5; 20 minutes, vortexed at five minutes interval at 37°C). The oocysts were centrifuged (3000 g for four minutes at 20°C), resuspended in parasite-maintenance Dulbecco's modified eagle's medium (DMEM; Gibco, Auckland, New Zealand) containing 4.5 g/L d-glucose, 0.58 g/L l-glutamine, 3.7 g/L sodium bicarbonate, 0.20 g/L bovine bile, 0.004 g/L folic acid, 0.001 g/L 4-aminobenzoic acid, 0.004 g/L d-calcium pantothenate, 0.88 g/L ascorbic acid, 1%

heat-inactivated FBS, 2.4 g/L HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 100,000 U/L penicillin, 100,000 mg/L streptomycin, and 250 mg/L amphotericin B, adjusted to pH 7.4), and incubated for three hours at 37°C. The supernatant was removed, fresh DMEM was added, and oocysts were kept at 37°C for 90 minutes. The final dose of oocysts was adjusted 5 000/well prior to inoculation and cell culture ([8]).

3.4. In Vitro Cultivation

3.4.1. Preparation and Infection of Host Cells

The cell line HT-29 was purchased from Iranian Biological Research Centre, Tehran. The HT-29 cells were harvested in 25-cm2 flasks, followed by subculture in 24-well plates, supplemented with DMEM containing 10% fatal bovine serum (FBS; Gibco, Auckland, New Zealand), 100 U/mL penicillin, 100μg/mL streptomycin, and 25 μg/mL amphotericin B incubated with 5% CO_2 at 37°C for 48 hours, and confluent monolayer cells were developed. For 24-well plates, the supernatant DMEM of the wells were removed and washed once with PBS (pH = 7.2). Next, 100 μL DMEM containing 5000 oocysts were inoculated onto each well, and the plates were incubated with 5% CO_2 at 37°C for six hours. The 24-well plates were also inoculated with 100 μL DMEM containing 5000 oocysts/cm2 and incubated with 5% CO_2 at 37°C for 24 hours. A 24-well plate was inoculated with PBS as control (mock). The HT-29 cell lines were exposed to *C. parvum* for 6 and 24 hours, and then, the cells were lysed with buffer RLT (RNeasy mini kit, Qiagen).

3.5. RNA Isolation and RT2 Profiler™ PCR Array

The total RNA was extracted for 6 and 24 hours separately using RNeasy mini kit (Qiagen), as per the manufacturer's instructions. The cDNA was prepared using RT2 first standard kit (Qiagen). The qPCR Array was performed using the RT2 Profiler™ PCR Array of SABiosciences (http://www.sabiosciences.com/ArrayList.php) according to the manufacturer's instruction. The triplicate test was conducted on each sample. Briefly, 2700 μL reaction mixture containing 1350 μL 2x RT2 SYBR Green qPCR Mastermix, 102μL cDNA, and 1248 μL RNAase-free water were prepared. The 25 μL of reaction mixture was added to each 96-well custom RT2 Profiler™ PCR Arrays and was subjected to real-time PCR (Applied Biosystems, Foster City, CA) with the following program: 95°C, 10 minutes, one cycle, 40 cycle, 15 seconds, 95°C, followed by 60°C for one minute (fluorescence data collection). The threshold cycle (Ct) for each well was calculated as described in web-based PCR Array Data Analysis Software (www.SABiosciences.com/pcrarraydataanalysis.php). A triplicate test was conducted to evaluate gene expression.

The gene expressions of the each sample were appraised on 84 genes (Table 1) related to human type I INFs (PAHS-016ZA). In addition, five housekeeping genes (*ACTB, B2M, GAPDH, HPRT1, PRLPO*), one genomic DNA control (GDC), three reversed transcription control (RTC), and three positive PCR control were used in the assay. The following overexpression and underexpression values were determined by fold change assessment. Fold-change (2ˆ [-delta delta Ct]) is the normalized gene expression (2ˆ(-delta Ct)) in the test sample divided by the normalized gene expression (2ˆ [-delta Ct]) in the control sample. Fold-regulation represents fold-change results in a biologically significant way. Fold-change values > 1 indicate a positive or upregulation; the fold-regulation is equal to the fold-change. Fold-change values > 1 indicate a negative or down-regulation, and the fold-regulation is the negative inverse of the fold-change.

3.6. Statistical Analysis

The results of overexpression and underexpression of each gene related to type I INFs were compared with mock cells using online Software (www.SABiosciences.com/pcrarraydataanalysis.php). In vitro, cellular assays were done in triplicate. Data were analyzed using student *t* test. Based on fold change, the overexpression of genes and underexpression of genes were determined for six and 24 hours after infection. P value < 0.05 was considered significant. The P values are calculated based on a Student *t* test of the replicate 2ˆ(-delta Ct) values for each gene in the control and treatment groups. The automatic selection from housekeeping genes with genes (HKG) panel with *ACTB* and *GAPDH* was conducted to evaluate the normalization of the test samples for six and 24 hours. This method automatically selects an optimal set of internal control/housekeeping/normalization genes for the analysis from the available HKG panel on the PCR array.

4. Results

4.1. Inoculate Cryptosporidium parvum Iowa Strain

The post-infected neonatal rat with *C. parvum* showed symptoms such as weakness, severe diarrhea, dehydration, and volume depletion three to four days after oral inoculation. The microscopy presence of *Cryptosporidium* oocysts in feces of the infected neonatal rats was stained by Ziehl-Neelsen. The amount of 522 ng/μL of the extracted RNA from exposed cells to *C. parvum* was used for RT² PCR™ Array.

4.2. Gene Expression Pattern of Human Type I INF Response at RT² Profiler™ PCR Array

Based on technical triplicate data, fold change for the significant upregulation of the expressed gene was defined (fold-change > 1.0: P < 0.05) for six and 24 hours and fold change for significant downregulated gene was described (fold-change < 1.0; P < 0.05) following the infection. It should be noted that the GSEA analysis confirmed all the overexpressed genes related to type I INFs pathway.

4.3. Overexpressed, Underexpressed, and Unchanged Genes Six Hours Post-Infection

Four top overexpressed genes related to type I INFs namely *HLA-A, IL-10, MX1*, and *SH2D1A* were selected (Table 2 and Figure 1) and out of 60 downexpressed genes, five genes including *IRF2, IRF5, PML, TIMP1*, and *TYK2* were chosen for further investigation (Table 3 and Figure 1). The remaining 55 underexpressed genes were as follows: *CAV1, TAP1, TLR9, CD80, JAK1, IFI30, IFI27, IFITM2, SHB, OAS2, IFI6, IFNAR2, BST2, ADAR, STAT2, MET, PRKCZ, IRF9, IFNAR1, MX2, TICAM1, SOCS1, JAK2, IFI16, TRAF3, STAT1, BAG3, TLR3, ISG15, TNFSF10, IRF3, IFNA2, HLA-G, CXCL10, ISG20, STAT3, CASP1, IRF7, MYD88, NMI, IL15, IFITM3, IFNE, IFNA1, IRF1, IFITM1, IFNW1, IFIT1, IL6, IFIT3, IFIH1, NOS2, IFIT2, CIITA*, and *CCL5*. Twenty genes residual unchanged were as follows: *CCL2, CD70, CD86, CDKN1B, CRP, DDX58, EIF2AK2, GBP1, HLA-B, HLA-E, IFNA4, IFNB1, MAL, MNDA, OAS1, PSME2, TLR7, TLR8, TMEM173*, and *VEGFA*.

Table 2. Overexpressed Genes for 6 h (Fold-change > 1; P < 0.05)

Overexpressed Genes	Fold-change 6 h	P-value h
HLA-A	4614.27	0.019548
IL10	6.43	0.031975
MX1	20709.49	0.019887
SH2D1A	5.74	0.003034

Table 3. Underexpressed Genes for 6 h (Fold-change < 1; P < 0.05)

Under expressed Genes	Fold change 6 hours	P value hours
IRF2	-31.44	0.037850
IRF5	-801.25	0.028377
PML	-1977.58	0.001647
TIMP1	-2343.40	0.008456
TYK2	-8.34	0.042893

4.4. Overexpressed, Underexpressed, and Unchanged Genes 24 Hours Post-Infection

Sixty-seven genes were overexpressed among which 10 top genes such as *CASP1, CXCL10, DDX58, IFIH1, IFNAR2, IL15*,
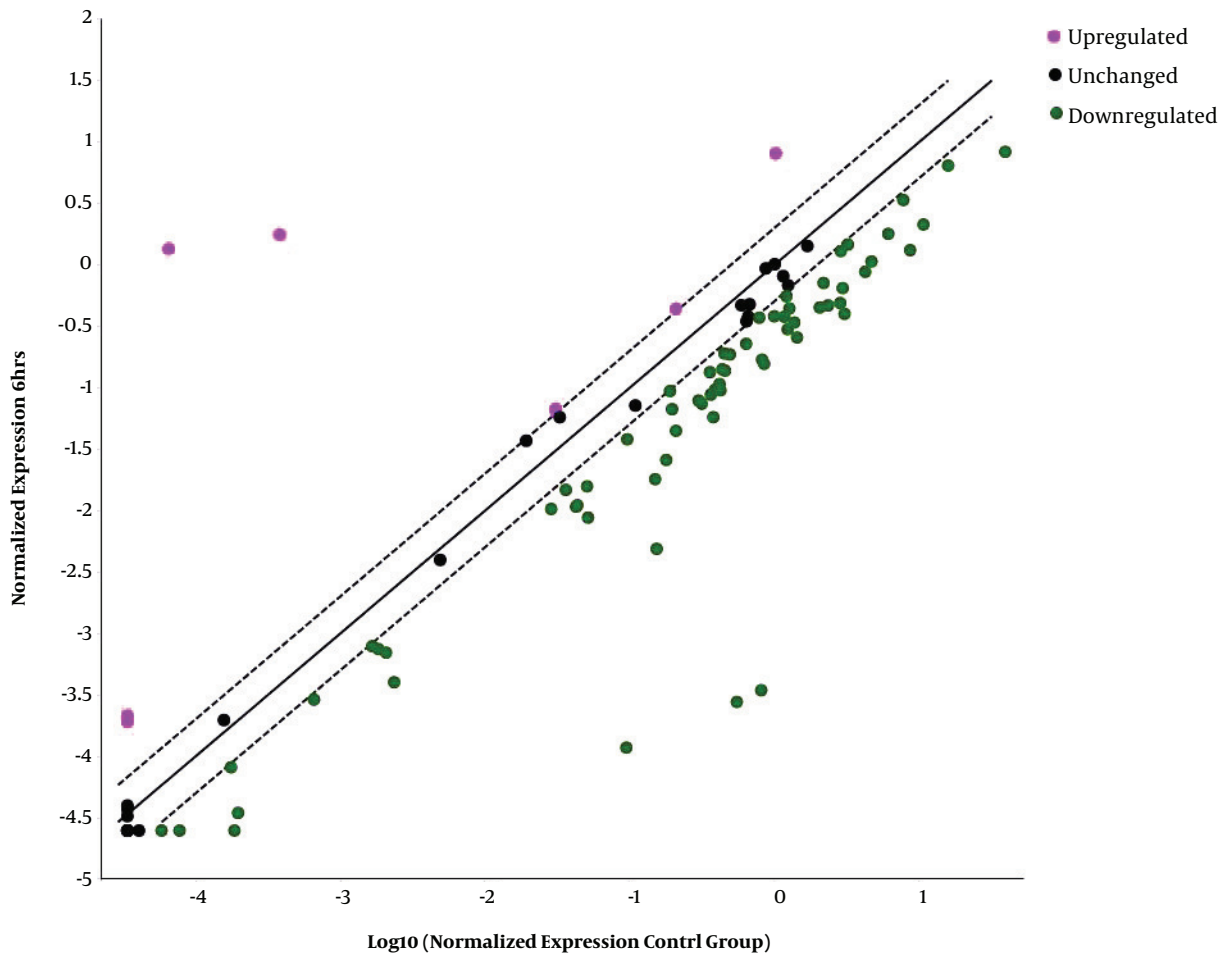
Figure 1. The scatter pattern shows overexpressed and downexpressed genes in infected HT-29 cells with *Cryptosporidium parvum* oocysts for 6 hours.

MYD88, NMI, STAT3, and *TLR3* were selected for further studies (Table 4, Figure 2). The following 57 overexpressed genes were as follows: *IFNE, IFNB1, CAV1, EIF2AK2, CDKN1B, ISG15, CCL5, ISG20, BAG3, PRKCZ, GBP1, IFNAR1, IFITM3, TIMP1, STAT1, HLA-A, VEGFA, JAK1, IFIT2, IFIT3, ADAR, TRAF3, IFNA1, OAS2, HLA-E, TMEM173, IRF2, IFITM2, IFI16, IFI27, SOCS1, MX2, PML, TYK2, SHB, OAS1, MX1, IFI30, CIITA, BST2, NOS2, IRF7, IRF1, CD70, TN-FSF10, STAT2, TICAM1, IRF9, IRF5, IFNW1, IFI6, IFNA4, TAP1, CCL2, MNDA, IFNA2*, and *IL6*. Three underexpressed genes *IFITM1, PSME2*, and *IRF3* were observed in post-infected cells. The remaining 14 unchanged genes related to type I INFs were diagnosed in the infected cells as follows: *TLR7, TLR8, TLR9, SH2D1A, JAK2, MAL, MET, IL10, IFIT1, HLA-B, HLA-G, CRP, CD80*, and *CD86*. The cluster gram pattern showed that the overexpressed and underexpressed genes were related to human type I INF after six and 24 hours exposure (Figure 3 A & B).

Table 4. Overexpressed Genes for 24 h (Fold-change > 1; P < 0.05)

Overexpressed Gene	Fold-change 24 h	P-value 24 h
CASP1	12.46	0.008962
CXCL10	21.76	0.002424
DDX58	50719.61	0.008805
IFIH1	14.42	0.002223
IFNAR2	14.50	0.002332
IL15	16.48	0.022768
MYD88	16.37	0.009969
NMI	17.81	0.000123
STAT3	15.13	0.011018
TLR3	12.05	0.002110

5. Discussion

It is important to understand whether the reaction of exposed cells to *C. parvum* cells are resistant or the disease
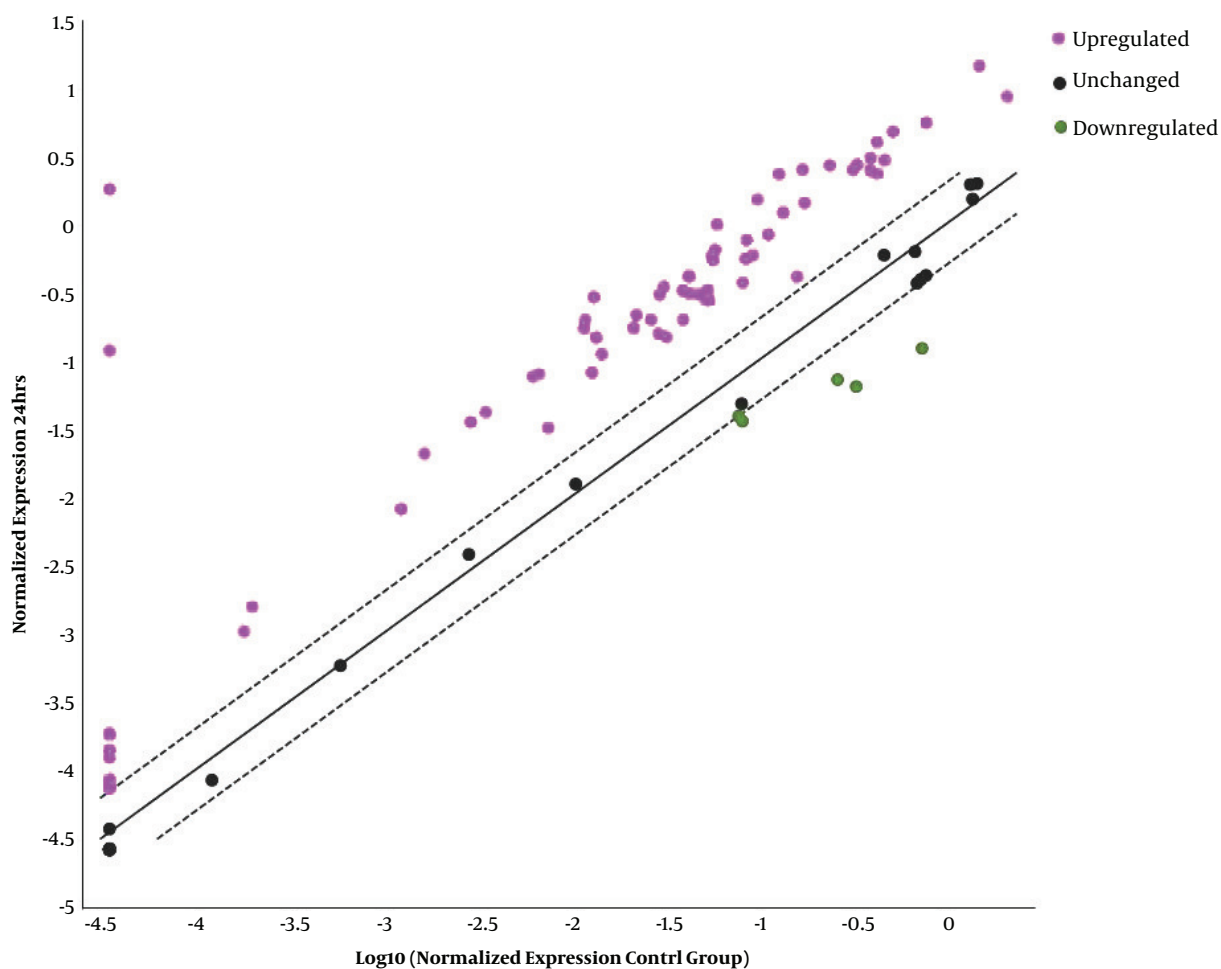
Figure 2. The scatter pattern shows overexpressed and downexpressed genes for 24 hours after infection mock cell (control) and stimulated cell of HT-29 with *Cryptosporidium parvum* oocysts.

develops. Therefore, it is important to evaluate the innate immune genes related to type I INF response. Limited information is available to describe immune response expression of genes in cells exposed to *C. parvum*. Therefore, the interest of the current study was to find out overexpression and underexpression of related genes to human type I INFs (α and β) in HT-29 cells exposed to *C. parvum* for six and 24 hours. Based on fold-change outcomes, four top overexpressed genes with fold-change > 1.0 (P < 0.05) including *HLA-A* and *MX1* genes were evaluated in infected cells for six hours. Birte Pantenburg et al., reported that the *HLA-A* and *-B* were involved in presenting the *C. parvum* antigens to CD8 + T-lymphocytes to eliminate the parasite ([8]). The current study found that the *HLA-A* gene was overexpressed in infected cells after six hours exposure. This finding was in agreement with what Birte Pantenburg et al., reported.

IFN-α and -β induce *MX1* gene expression that encodes a GTPase activity and acts as a potent inhibitor for replication of many RNA viruses and *Salmonella typhimurium* ([9-11]). The current study data showed that MX1 gene was over expressed in the exposed cell six hours post-exposure. It was thought-provoking to express the *MX1* gene as an antiviral factor induced by a *C. parvum* protein, which needs further investigation. Expression of *SH2D1A* gene synthesizes protein 2B4, which can interact with SLAM (signaling lymphocyte-activating molecule) to monitor T-cell and B-cell response to Epstein-Barr virus (EBV) infection ([12-14]). The current study findings revealed that *SH2D1A* gene was overexpressed in vitro for six hours. It is necessary to investigate the characterization of antigen or protein that induces SH2D1A gene expression against *C. parvum* infection. Wyatt et al., found that *C. parvum* epitopes (44 to > 200 kD) could prompt overexpression of *IL-10* gene in intestinal ep-
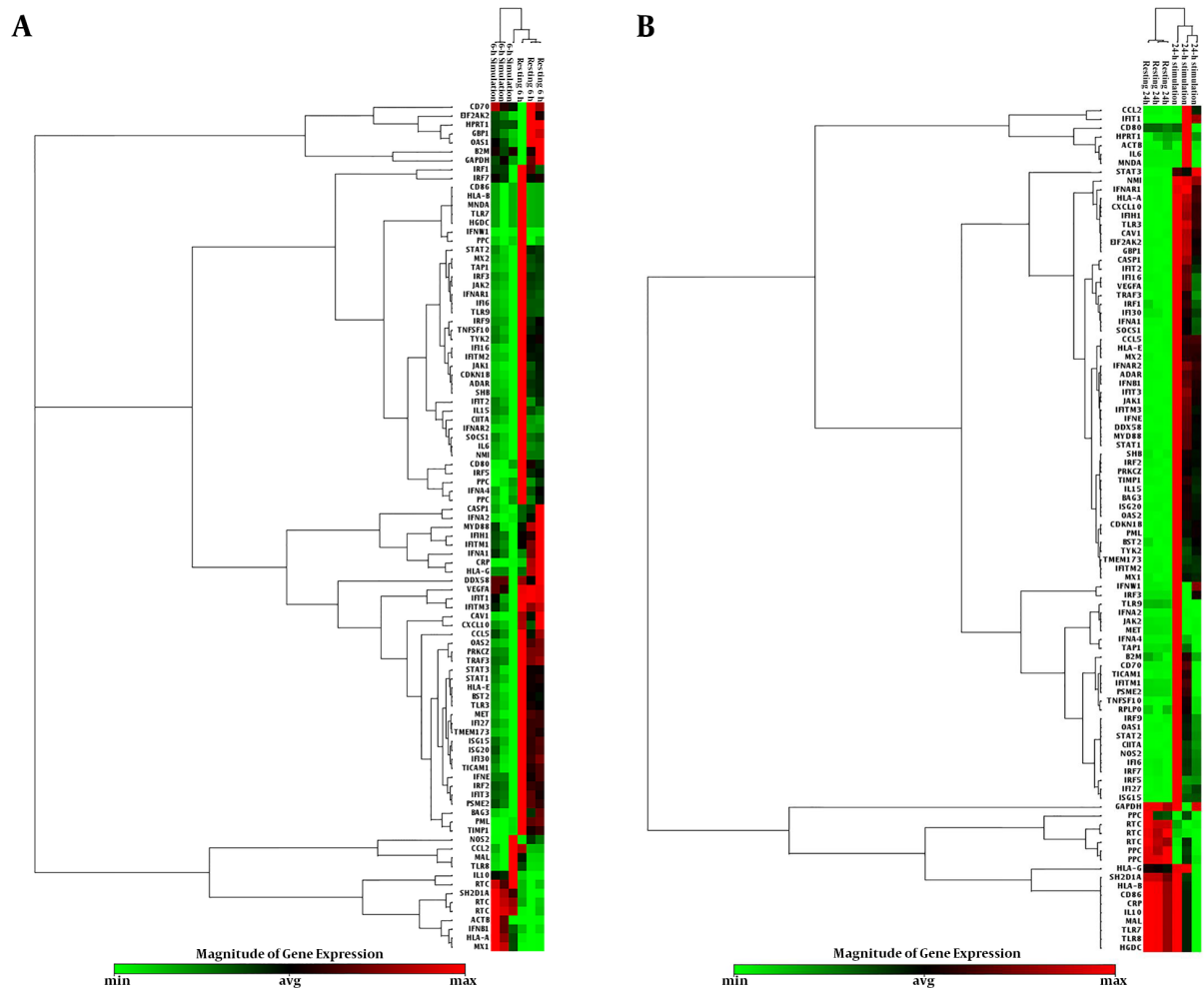
A

B

Magnitude of Gene Expression

min   avg   max

Figure 3. The clustergram pattern shows overexpressed and underexpressed genes related to human type I interferon response in infected cells and mock cells for 6 (A) and 24 hours(B).

ithelial cells. They concluded that the expression of *IL-10* gene may have an important role in developing parasite infection (15). Based on the current study data, *IL-10* gene was overexpressed in the infected cells.

The following genes, *TIMP1, TYK2, IRF2, PML*, and *IRF5* were under expressed in the infected cells for six hours. The expression of *TIMP1* (tissue inhibitors of metalloproteinases) gene inhibits expression of *MMPs* (matrix metalloproteinases) gene, which is implicated in developing cancer, neurological, and infectious pathologies (16). Overexpression of *TIMP1* gene is implicated in clearance of parasites infection (16, 17). According to the current study findings, the *TIMP1* gene was underexpressed for six hours, but overexpressed in the infected cells for 24 hours. Thus, it was concluded that the overexpression of *TIMP1* and *SH2D1A* genes might be an important key for *C. parvum* to become

self-limited infection.

The expression of *TYK2* gene plays a significant role in the intracellular signaling of type I IFNs using phosphorylation and activation of STAT proteins (18). The expression of *TYK2* gene plays a vital role in the synthesis of INF-β. The stimulation of INF-β production via overexpression of *TYK2* gene is detected in the infected cells by *L. major*. The current study observed that *TYK2* gene was downexpressed for six hours, but overexpressed for 24 hours following the infection. In contrast, Piyal Ganguli stated that leishmaniosis stimulated the production of INF-β, which in turn can upregulate the secretion of RAP1 and SOCS3 proteins within the T-cells that potentially inhibits MAPK and JAK-STAT signaling pathway via TYK2 mediated pathway (19).

The expression of *IRF2* (interferon regulatory factor 2) gene upregulated type I INF associated with signaling path-

way and resulted in the synthesis of IL-12 in cells infected by *L. major* (20, 21). *IRF2* protein acts multiple functions such as transcription factor, *IRF2*, transcriptional activator of histone H4, IFN-stimulated regulatory element (ISRE)-responsive genes and requires optimal maturation of natural killer (NK) cells (22). The current study recorded the IRF2 gene as underexpressed for six hours, but it was overexpressed for 24 hours. Thus, it is very important to investigate the characterization of a protein of *C. parvum* to induce *IRF2* gene expression.

Promyelocytic leukemia (*PML*) gene produces a protein, acts as a tumour suppress and prevents cells from growing and dividing. Promyelocytic leukemia protein is also a cofactor to induce apoptosis. In the current study, the *PML* gene was downexpressed in the infected cells for six and 24 hours. TLR 7, TLR 9 via signaling pathway of INF type I induced *IRF5* gene expression, which activates pro-inflammatory cytokines such as *IL6* and *TNF* (tumor necrosis factor) (23, 24). The expression of *IRF5* gene was detected in the infected cells by *L. donovani* (25). The current study data showed that *IRF5* gene was downexpressed in the infected cells for six and 24 hours. The current study selected 10 top overexpressed genes with high enrichment including *IL15, DDX58, CXCL10, NMI, MYD88, STAT3, IFNAR2, IFIH1, CASP1*, and *TLR3* genes relevant to types I IFNs in post-infected cells for 24 hours. IL-15 (mRNA is overexpressed in cells of secretary tissues, dendritic cells (DCs), epithelial cells, bone marrow stromal cells, and fibroblasts) and its functions stimulate the proliferation of T-lymphocytes and important role of *IL-15* to control infection (26-28). The current study results indicated that *IL-15* gene was overexpressed in the infected cell for 24 hours; to the authors' best knowledge, an overexpression of *IL-15* is reported in infected cells by Cryptosporidiosis.

The expression of *DDX58* gene can promote immunological responses (29). Alvarez et al., reported that *DDX58* gene was downexpressed in lamb liver tissue cells infected by *Fasiola hepatica* (30). In contrast, the current study found that *DDX58* gene was overexpressed in the infected cells for 24 hours. *CXCL10* is a Chemokine and can attract the immune cells at the site of infection, while in the patients with AIDS, due to lack of immune cells, *CXCL10* is enabled to recruit immune inflammatory cells causing immune pathogenesis. Carneiro et al., reported that the genes related to IFN-γ such as *CXCL10* were upregulated when HPs and LPs (high or low IFN-γ producers) following motivation of peripheral blood cells were exposed to *L. braziliense*, which was in line with the current study findings (31). The expression of *CXCL10* gene via stimulation of *IFN-δ* gene was detected in intestinal epithelial cells infected by *C. parvum* (32). Type I IFN and IFN-γ stimulated the expression of N-myc interactor (*Nmi*) gene (33). NMI

is a cytokine with a multiple function including enhancement *IFN-γ*-induced STAT-dependent transcription and increased interleukin-2 (*IL-2*)-driven STAT5-dependent relative transcription (34). NMI protein acts as antiviral activities against prototype foamy virus (35), Sendai virus (33), and foot and mouth disease virus (FMDV) (36). To the authors' best knowledge, parasite induced NMI protein is not reported yet. In the current study, the *NMI* gene was overexpressed in the infected cells for 24 hours.

MyD88 gene encodes a protein and implicates in innate immunity. Toll-like receptor 4 induced type I INF via *MYD88* (37). Lacroix-Lamande et al., injected polyC to neonatal mice infected with *C. parvum*, and found enhancement signalling MYD88 pathway, which resulted in resistance of mice intestine cells to parasite oocysts infection (38, 39). Rogers et al., reported that mice lacking *MyD88* were more at risk to infection than the wild-type mice (38). In the current study, MYD88 gene was overexpressed for 24 hours indicating an important key role in activation of type I INF. The TLR3 protein performs multiple functions such as pathogen recognition, activation of innate immunity, recognition of dsRNA associated with viral infection, and inducing NF-β and type I INF production (40). The current study observed that the *TLR-3* gene was overexpressed for 24 hours in vitro, which was in agreement with the findings of Koblansky et al., (41) but in contrast with those of the study by Beiting (42). *STAT3* is a transcription factor that can be activated by interferon ligand that implicates in apoptosis and cell growth processes (43). Kyeong et al. showed that STAT3 gene was downexpressed in Caco-2 cells infected with *Entamoeba histolytica*, but it was in contrast with the current study results. The *STAT3* gene was overexpressed for 24 hours (44).

Caspase-1, which can activate two inflammatory cytokines and initiate the pro-apoptosis program, results in cell death (45). Liu et al., reported that about 20% of infected cells led to apoptosis for 48 hours, but the majority of cells were protected from apoptosis by *C. parvum* infection. They found high levels of *caspase-3, -4, -6*, and *-7* activation and low levels of *caspase-2, -8*, and *-9* activation, while no *caspase-1* and *-10* genes were expressed at a 48-hour course of infection. *Cryptosporidium parvum* suppressed staurosporine-induced apoptosis and initiated caspase-3/7 activation of intestinal epithelial cells via upregulation of *survivin*, which sustains parasite infection (46). The current study found that the *caspase-1* gene was overexpressed for 24 hours. In the current study, *caspase-2-10* gene expressions were not evaluated, even though it was necessary for further study.

IFNAR2 (interferon-alpha/beta receptor beta) protein bands activate the receptor stimulation Janus protein kinases, which in turn activate *STAT1* and *STAT2*. It sub-

sequently produces type I IFN and pro-inflammatory cytokines, in that way up regulating a family of IFN-stimulated genes (*ISGs*) that exert pleiotropic inhibitory effects on viral reproduction in the neighboring cells (47, 48). The overexpression of *IFNAR2* gene is yet to be reported in the infected cell by *C. parvum*. The current study found that the *IFNAR2* gene was overexpressed in the infected cells for 24 hours, even though its functions should be investigated.

IFIH1 (interferon-induced helicase 1) recognizes dsRNA of many viruses and acts as mediator for antiviral response (49). The *IFIH1* gene was overexpressed, but its function requires further evaluation. The cells infected by *C. parvum* result in overexpressed genes related to subtype 1 interferon including synthesis of MX1, SH2D1A, TLR3, NMI, and IFIH1 proteins, which may have antiviral properties and are promising new candidate biological drugs against some viral infections. Han et al., reported that anti-viral cytokine interferon-β (IFN-β) was produced after IMs (inflammatory monocytes) exposed to *Toxoplasma gondii* (50).

6. Conclusions

In conclusion, the overexpression of *TIMP1* gene had an important role in the clearance of *C. parvum*. The overexpression of *TIMP1, SH2D1A*, and *MYD88* genes may lead *C. parvum* to become a self-limited infection. The overexpressed *TIMP1* may have anticancer properties. The overexpression of *TYK2* and *MYD88* genes synthetizing type I INF may play an important role in innate immunity. It is important to investigate the induction of production the protein and overexpression of *IRF2* gene with multiple functions related to subtype I INF. The *CXCL10* gene function is involved in the attraction of immune effector cells to the site of infection, the overexpressed *CXCL10* was observed. The current study identified the upregulated genes, which can prevent the invasion of parasite to cells. Finally, the identification of all unknown induced overexpressed and underexpressed genes by *C. parvum* requires thorough investigation. In the light of aforementioned overexpressed genes related to type I IFN as a part of innate immunity may play an important role against *C. parvum* infection.

Acknowledgments

Footnote

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Table 1. The Profile of Human Genes Related to Type I Interferon by RT^2 Profiler™ PCR Array

Gene Symbol	Description
ADAR	Adenosine deaminase, RNA-specific
BAG3	BCL2-associated athanogene 3
BST2	Bone marrow stromal cell antigen 2
CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
CAV1	Caveolin 1, caveolae protein, 22 kDa
CCL2	Chemokine (C-C motif) ligand 2
CCL5	Chemokine (C-C motif) ligand 5
CD70	CD70 molecule
CD80	CD80 molecule
CD86	CD86 molecule
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)
CIITA	Class II, major histocompatibility complex, transactivator
CRP	C-reactive protein, pentraxin-related
CXCL10	Chemokine(C-X-C motif) ligand 10
DDX58	DEAD(Asp-Glu-Ala-Asp) box polypeptide 58
EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2
GBP1	Guanylate binding protein 1, interferon-inducible
HLA-A	Major histocompatibility complex, class I, A
HLA-B	Major histocompatibility complex, class I, B
HLA-E	Major histocompatibility complex, class I, E
HLA-G	Major histocompatibility complex, class I, G
IFI16	Interferon, gamma-inducible protein 16
IFI27	Interferon, alpha-inducible protein 27
IFI30	Interferon, gamma-inducible protein 30
IFI6	Interferon, alpha-inducible protein 6
IFIH1	Interferon induced with helicase C domain 1
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
IFITM1	Interferon induced transmembrane protein 1(9–27)
IFITM2	Interferon induced transmembrane protein 2(1–8D)
IFI	Interferon induced transmembrane protein 3
IFNA1	Interferon, alpha 1
IFNA2	Interferon, alpha 2
IFNA4	Interferon, alpha 4
IFNAR1	Interferon (alpha, beta and omega) receptor 1
IFNAR2	Interferon (alpha, beta and omega) receptor 2
IFNB1	Interferon, beta 1, fibroblast
IFNE	Interferon, epsilon

IFNW1	Interferon, omega 1
IL10	Interleukin 10
IL15	Interleukin 15
IL6	Interleukin 6 (interferon, beta 2)
IRF1	Interferon regulatory factor 1
IRF2	Interferon regulatory factor 2
IRF3	Interferon regulatory factor 3
IRF5	Interferon regulatory factor 5
IRF7	Interferon regulatory factor 7
IRF9	Interferon regulatory factor 9
ISG15	ubiquitin-like modifier
ISG20	Interferon stimulated exonuclease gene 20kDa
JAK1	Janus kinase 1
JAK2	Janus kinase 2
MAL	Mal, T-cell differentiation protein
MET	Met proto-oncogene(hepatocyte growth factor receptor)
MNDA	Myeloid cell nuclear differentiation antigen
MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78(mouse)
MX2	Myxovirus (influenza virus) resistance 2(mouse)
MYD88	Myeloid differentiation primary response gene(88)
NMI	N-myc (and STAT) interactor NM_ NOS2 Nitric oxide synthase 2, inducible
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa
PML	Promyelocytic leukemia
PRKCZ	Protein kinase C, zeta
PSME2	Proteasome(prosome, macropain) activator subunit 2(PA28 beta)
SH2D1A	SH2 domain containing 1A
SHB	Src homology 2 domain containing adaptor protein B
SOCS1	Suppressor of cytokine signaling 1
STAT1	Signal transducer and activator of transcription 1, 91kDa
STAT2	Signal transducer and activator of transcription 2, 113kDa
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
TAP1	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
TICAM1	Toll-like receptor adaptor molecule 1
TIMP1	TIMP metallopeptidase inhibitor 1
TLR3	Toll-like receptor 3
TLR7	Toll-like receptor 7
TLR8	Toll-like receptor 8 Toll-like receptor
TMEM173	Transmembrane protein 173
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10
TRAF3	TNF receptor-associated factor 3
TYK2	Tyrosine kinase 2
B2M	Beta-2-microglobulin

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HPRT1	Hypoxanthine phosphoribosyltransferase 1
RPLP0	Ribosomal protein, large, P0
HGDC	Human genomic DNA contamination
RTC	Reverse transcription control
RTC	Reverse transcription control
RTC	Reverse transcription control
PPC	Positive PCR control
PPC	Positive PCR control
PPC	Positive PCR control