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Human Immune-Inflammatory Gene expression Profile after Infection with Human Papillomavirus

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Abstract: Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease worldwide in both men and women. HPV is associated with a variety of diseases ranged from innocuous lesions to cancer. Three Data-groups of up regulated immune-inflammatory genes (25 genes with 3 replicates) that affected by human papilloma virus especially types 16, 18, 31, and 45 were subjected to this study. Bioinformatics is an emerging scientific discipline used in this study to organize, analyze, and distribute biological information of genes affected by HPV. Data analysis of Real-time-reverse transcription PCR-array (RT-PCR-array) is the most sensitive and reliable method used to analyze the human gene expression affected by HPV. Gene ontology is a new bioinformatics method mainly provides typical batch annotation and gene analysis used to highlight the most important functions of up regulated genes after HPV infections. The immune-inflammatory gene expression profile for host affected with HPV was: 25 up regulated genes with 6.47 fold change (CCL18) to79.22 (IL1RN).

Keywords: Human papilloma virus, Gene expression, Real-Time-PCR Array, Bioinformatics, Gene Ontology.

Introduction

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease worldwide in both men and women and is thought to be the most common sexually transmitted viral disease. Papillomaviruses are ubiquitous and have been detected in a wide variety of animals as well as in humans and are specific for their respective hosts. More than 200 types of HPV have been recognized on the basis of DNA sequence data showing genomic differences (Burd, 2003).HPV is associated with a variety of clinical conditions that range from innocuous lesions to cancer. Most HPV infections can cause warts (plantar warts, common warts and flat warts). Genital HPV types infect primarily the cervix, vagina, vulva, penis and anus. Among the cancers attributable to high-risk HPV infection, cervical cancer has received the most attention. HPV-16, -18, - 31, -45 accounts for more than 90% of cervical carcinomas. Of these types, HPV-16 is the most often found, accounting for about half of the cervical cancer cases in the United States and Europe. In addition, high-risk HPV types have been related with other genital cancers, such as carcinoma of vagina, vulva, penis and anus, and their precancerous lesions. (Gomez and Santos, 2007)
Bioinformatics is an emerging scientific discipline that uses information technology to organize, analyze, and distribute biological information in order to answer complex biological questions (Abd-Elsalam 2003). Microarray analysis and RT-qPCR are powerful methods to determine global profiles of genes in cells and tissues under a variety of complex biological conditions(Clewley, 2004). Development of microarrays facilitated the screening of viral pathogens from across broad viral families (See and Work, 2008; Penelope, et al., 2004). Data analysis of Real-time-reverse transcription PCR-array (RT-PCR-array) (SABioscience) is the most sensitive and reliable method for gene expression analysis (Montgomery and Daum 2009) which can be used toanalyze the results of previous studies (Performed on the effect of HPV on human immune genes) through the determination of the altered genes and connect them with the new advanced technique(Gene Ontology) which provides the scientific researcher with new and important information mainly provides typical batch annotation and gene analysis to highlight the most relevant pathways associated with a given gene list (Huang et al., 2009). In addition Gene Ontology can determine the sit effect of these altered genes on the most important immune-inflammatory pathways in patients affected with microbial infections (David, 2008). The aim of this study is to determine thehuman immune-inflammatory gene expression profile after infection with human Papillomavirus especially cancerous types 16, 18, 31, and 45. In addition to specify the up regulated genes and their function during HPV infections

Materials and Methods

Gene-Data Collection

Three groups of up regulated immune-inflammatory genes were collected from previous studies after infection with cancerous human papilloma virus especiallytypes 16, 18, 31, and 45. The collected genes that their gene expression changes (with different fold change) were classified into three replicates and regarded as the Acute sample (Table 1).

Table 1: Fold change of gene expression and replicates of immune-inflammatory genes after HPV infections.

		Up regulated genes	Fold change of infected genes by HPV		
Gene No.	Position	Symbol	Group1	Group2	Group3
1	A02	BCL6	2.0	2.5	3.0
2	A11	CCL17	2.0	2.07	2.4
3	A12	CCL18	1.8	1.5	2.0
4	B01	CCL19	1.8	1.9	2.0
5	B02	CCL2	2	3.1	4.3
6	B03	CCL20	2.0	2.4	3.6
7	B04	CCL21	2.0	5.9	9.8
8	B12	CCL7	2.3	9.3	5.8
9	C08	CCR7	2.0	2.1	2.0
10	C11	CEBPB	2.0	2.4	4.8
11	D02	CXCL1	3.7	7.9	12.1
12	D04	CXCL11	2.3	3.1	3.5
13	D12	CXCL9	1.9	2.0	2.6
14	E02	IFNA2	2.0	4.8	8.9
15	E03	IL-10	2.0	4.6	6.0
16	E04	IL10RA	2.0	13.0	24
17	E08	IL17C	1.6	2.0	2.1
18	E09	IL-1A	2.0	2.1	5.8
19	E10	IL-1B	1.5	2.0	3.6
20	F04	IL36G	2.0	6.9	11.0
21	F06	IL1RN	1.61	6.6	11.6
22	F10	IL-8	2.0	2.5	2.1
23	G06	MIF	3.4	13.00	24.4
24	G09	TNF	1.8	2.0	2.5
25	G10	TOLLIP	2.8	3.3	4.5

The up regulated genes with more than one fold change were compared with control group (One foldchange) which has been taken from standard tables of previous studies (Al-Ghazal, 2010).

Calculation of fold change

The fold changes of genes samples were changed intothreshold values (Ct) which represent the initial cycle at which the DNA sample was amplified usingReal Time PCR-Array-DataAnalysis (Table 2).

Table 2: Fold changes values concluded from genes undergoing high expression afterHPV infections.

Gene name	**Group 1**	Ct Control	Ct Acute	**Group 2**	Ct Control	Ct Acute	**Group 3**	Ct Control	Ct Acute
BCL6	**2.0**	30.5	27.8	**2.5**	27.7	21.8	**3.0**	24.6	29.1
CCL17	**2.0**	30.5	27.8	**2.07**	30.5	27.8	**2.4**	32.7	29.7
CCL18	**1.8**	31.2	28.6	**1.5**	31.2	28.9	**2.0**	30.5	27.8
CCL19	**1.8**	31.2	28.6	**1.9**	27.4	21.9	**2.0**	30.5	27.8
CCL2	**2**	30.5	27.8	**3.1**	25.0	19.8	**4.3**	24.3	29.3
CCL20	**2.0**	30.5	27.8	**2.4**	32.7	29.7	**3.6**	29.1	32.4
CCL21	**2.0**	30.5	27.8	**5.9**	33.2	29.0	**9.8**	24.4	31.0
CCL7	**2.3**	27.7	23.6	**9.3**	24.1	30.4	**5.8**	29.1	33.3
CCR7	**2.0**	30.5	27.8	**2.1**	28.8	24.4	**2.0**	30.5	27.8
CEBPB	**2.0**	30.5	27.8	**2.4**	32.7	29.7	**4.8**	23.4	28.9
CXCL1	**3.7**	31.5	27.9	**7.9**	28.0	21.5	**12.1**	22.0	27.0
CXCL11	**2.3**	27.5	23.6	**3.1**	25.0	19.9	**3.5**	32.7	29.2
CXCL9	**1.9**	27.4	21.9	**2.0**	30.5	27.8	**2.6**	26.6	30.9
IFNA2	**2.0**	30.5	27.8	**4.8**	28.9	23.4	**8.9**	23.2	27.6
IL-10	**2.0**	30.5	27.8	**4.6**	27	21.3	**6.0**	23.7	29.6
IL10RA	**2.0**	30.5	27.8	**13.0**	33.6	28.2	**24**	24.0	29.0
IL17C	**1.6**	29.6	24.3	**2.0**	30.5	27.8	**2.1**	24.4	28.8
IL-1A	**2.0**	30.5	27.8	**2.1**	28.8	24.0	**5.8**	29.1	33.3
IL-1B	**1.5**	31.2	28.9	**2.0**	30.5	27.8	**3.6**	29.1	32.4

IL36G	**2.0**	30.5	27.8	**6.9**	31.3	25.7	**11.0**	25.0	31.3
IL1RN	**1.6**	29.6	24.3	**6.6**	31.3	25.7	**11.6**	20.6	27.7
IL-8	**2.0**	30.5	27.8	**2.5**	27.7	21.8	**2.1**	24.4	28.8
MIF	**3.4**	28.2	32.7	**13.0**	33.6	28.2	**24.4**	24.5	29.3
TNF	**1.8**	31.2	28.6	**2.0**	30.5	27.8	**2.5**	21.8	27.7
TOLLIP	**2.8**	24.6	21.7	**3.3**	23.3	26.4	**4.5**	24.2	29.2

Analysis of CT values

The Ct values introduced into gene analysis site (SABioscience-RT-PCR-Array using a Blank Excel Spread-sheet, SABioscience Data Analysis Template Excel File (Campeau et al., 2009; Myskiw et al., 2009). The results of this analysis represent the new fold change values (3 replicas) of gene expression compared with the same control group in all genetic analysis (Table 2). After gene analysis the value of gene expression for each gene was determined; more than one fold (compared with one fold for control genes) regarded as up regulated gene. Those with less than one fold regarded as down regulated gene.

Genes of RT-PCR-Array

The up regulated genes collected from previous studies will introduced in SABioscience-RT-PCR-Array technique. Using a Blank Excel Spread-sheet SABioscience Data Analysis Template Excel File which includes the following 84 immune genes: Cytokine genes, Cytokine receptors genes ,Chemokine receptors and other genes involved in inflammatory response.

Gene Ontology

For gene ontology analysisthe gene code number (Unigene or Refseq), of up regulated genes (Table-2),will interred in the data analysis using: David Functional Annotation Bioinformatics Microarray Analysis, Gene-annotation enrichment analysis, Functional annotation clustering, KEGG pathway mapping, and gene-disease association.

Statistical analysis

Since each gene has 6 values (3 values for acute samples and 3 values for control samples). All control values of all genes tested against all acute values and the differences between the two values were analyzed using the K- paired sample (Friedman test) to calculate significance degree between control and acute (P-value)

Result and Discussion

The results showed that HPV especially the cancerous types cause different changes in the expression of 25 human immune-inflammatory genes in different studies ($p<0.021$). These changes classified into three groups (Table 2). First group inclued genes scattered randomly with different values ranged between 6.4 fold change in IL1B to 59.7 fold changeinCXCL9 gene (figure 1). This means that HPV cause moderate impact on host human cell and stimulate gradual expression of 25 different human immune-inflammatory genes (figure 2).

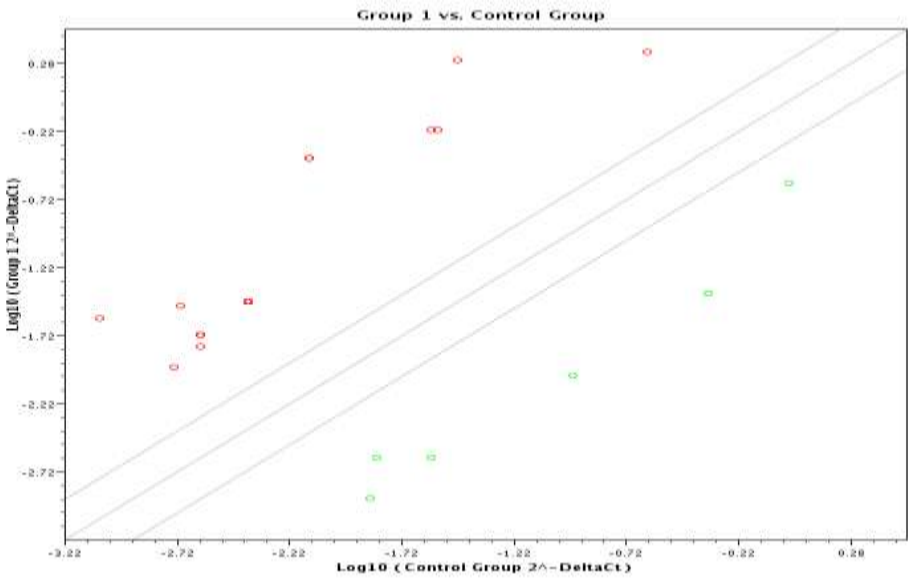

Figure 1: Scattered plot: Up regulated (Red circles) and down regulated (Green circles) genes in Acute 1 (Some circles may express more than one gene with same fold change).

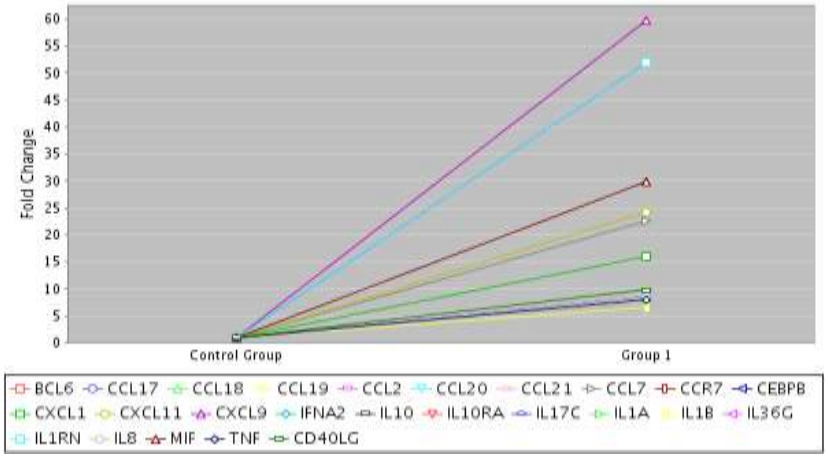

Figure 2: Multi curve: Up regulated genes (25 genes) for Acute 1

The second group of up regulated genes showed higher expression of genes ranged between 6.4 fold changes in4 genes (TNF, IL1B,IL17C, and CXCL9) and 90.5fold changes in CXCL1 gene (Figure 3). These results gave indicator that the effect of HPV on gene expression may vary according to the different types of HPV and to the immune status for different patients subjected to different studies (Figure 4).

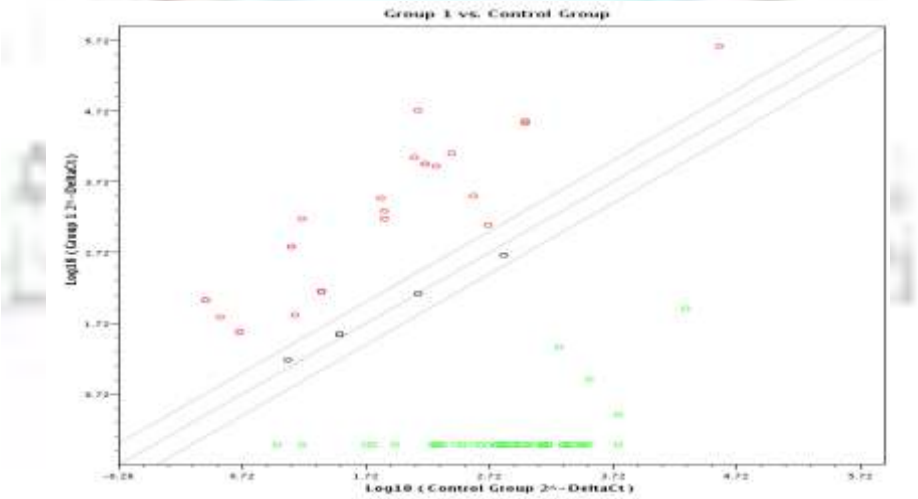

Figure 3: Gene analysis of Acute group 2 with 25 affected genes after HPV infections

Scattered plot: Up regulated (Red circles) and down regulated (Green circles) genes in Acute 2 (Some circles may express more than one gene (genes with same fold change).

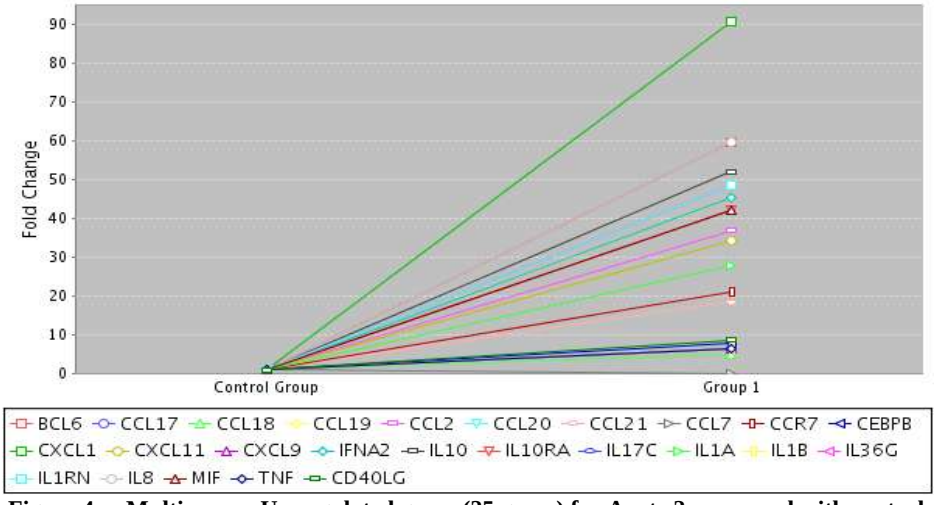

Figure 4: Multi curve: Up regulated genes (25 genes) for Acute 2 compared with control

The highest effect of HPV on the expression of human immune-inflammatory genes was revealed in the third group (Figure 5). The expression ranged between 6.4 fold change inCCL18 and 137.187inIL1RN which represents the most affected gene after infection with HPV(Figure 6).

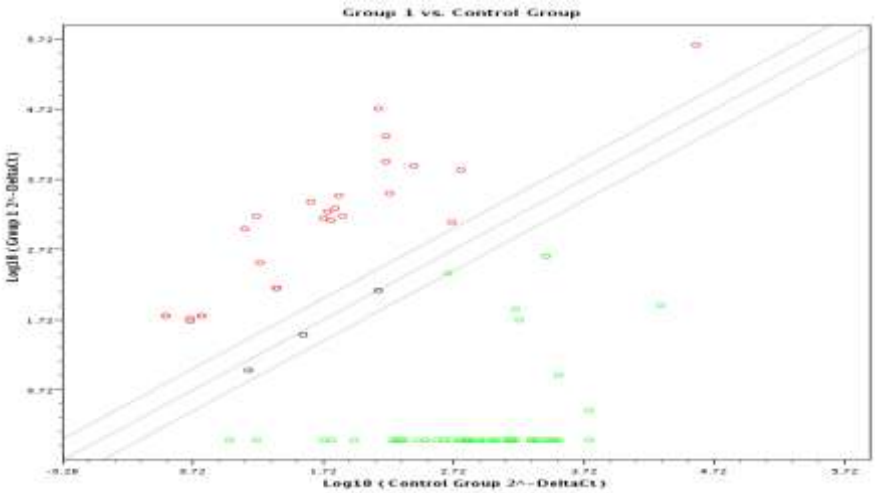

Figure 5: Gene analysis of Acute group 3 with 25 affected genes after HPV infections. Scattered plot: Up regulated (Red circles) and down regulated (Green circles) genes in Acute 3 (Some circles may express more than one gene (genes with same fold change.

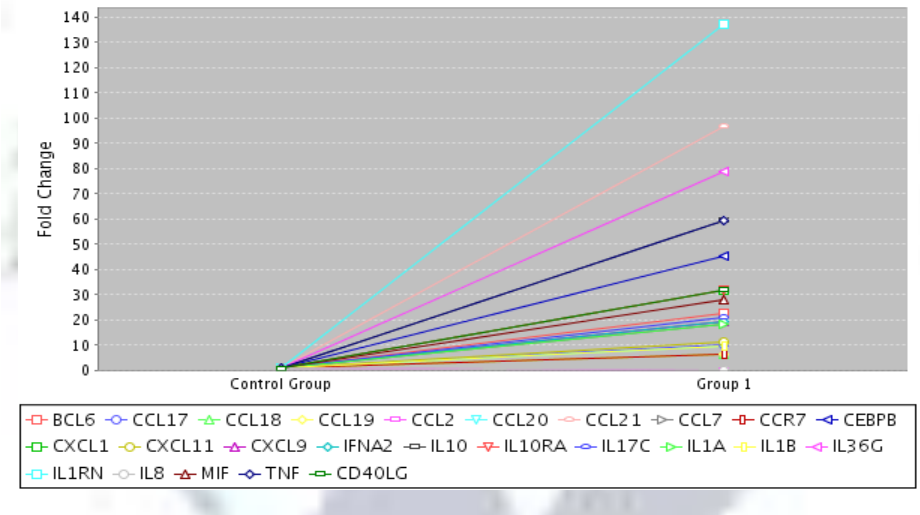

Figure 6: Multi curve: Up regulated genes (25 genes), for Acute 3.

The results showed that the most common affected genes were 11, those with mean of 22.77 to 79.22 fold changes in their expression (Table 3).

Table 3: The most important up regulated genes (22.77to 79.22 fold change) affected after HPV infection

	Gene symbol	Group1	Group2	Group3	Fold change (mean)
1	IL1RN	51.9842	48.5029	137.187	79.22
2	CXCL1	16	90.5097	32	46.16
3	IL36G	8.5742	48.5029	78.7932	45.28
4	CCL21	8.5742	18.3792	97.0059	41.31
5	IL10	8.5742	51.9842	59.7141	40.08
6	MIF	29.8571	42.2243	27.8576	33.30
7	BCL6	8.5742	59.7141	22.6274	30.3
8	CXCL9	59.7141	6.498	19.6983	28.63
9	IL10RA	8.5742	42.2243	32	27.59
10	IL17C	51.9842	6.498	21.1121	26.52
11	IFNA2	8.5742	45.2548	19.6983	22.77

Bioinformatics analysis using David Functional Annotation Bioinformatics Microarray Analysis, revealed that these up regulated 11 genes by HPV were mediated important inflammatory functions in human represented by: immune system processes, response to stimuli, locomotion, multi-organism process, developmental process, biological regulation.Intracellular Interleukin-1 Receptor Antagonist (IL1RN) was increased with 79.22 fold change which is more than other study (Chevalier et al.,2013) which also showed increasing in the fold change. Over expression of this gene is very important because it stimulates production of protein which inhibits the activities of interleukin 1, alpha (IL1A) and interleukin 1, beta (IL1B), and modulates a variety of interleukin 1 related immune and inflammatory responses (Szaboet al., 2010).

The mRNA transcript of Chemokine (C-X-C motif) ligand 1 (CXCL1) produced with 46.16 fold after HPV which regarded as a high level compared with other study (DeVotiet al., 2008) which showed 3.1 fold change only. Up regulation of this gene reflected its immunological roles such as stimulation the expression of beta-defensin which leads to antimicrobial barrier formation. In addition acts as a chemoattractant for neutrophils (Nischalkeet al., 2012). Interleukine-36 gamma gene (IL36G) (also called IL-1F9)increased with 45.28 fold change whereas in other study increased up to 13.1fold change (DeVotiet al 2008). This increasing was stimulated by HPV infection and immune system activates signaling transduction chainto activateslocal inflammatory response against HPV infections (Taylor et al., 2002). The expression of this cytokine in keratinocytes can also be induced by a contact hypersensitivity reaction or viral infection (Bachmann et al., 2012)

Chemokine ligand 21 (CCL21) increased with 41.31 fold change whereas in other study it was also increased but in low limit (more than 2 fold) (Trimble et al. 2010). This high increasing of this Chemokine is associated with, Inhibition of hemopoiesis, stimulation of chemotaxisthymocytes and activation of T-cells (Oliveira et al., 2013).. Interlukine-10 (IL-10) increased with 40.08 fold changes which in turn enhances B cell survival, proliferation, and antibody production (Zhenget al., 2012; Rezaeiet al., 2010). Many other studies also revealed that IL10 gene expression increased highly during infection and this high level of this protein enhance viral survive (Bermdez-Morales et al., 2011; Torres-Povedaet al., 2012; AL-Ghazal, 2010). The infection with HPV stimulates the host cells to activate expression of MIF gene (33.30 fold). In other study this gene also up regulated (14.352 fold change) (Savolaet al., 2011). The expression of MIF at sites of inflammation suggests a role as mediator in regulating thefunction of macrophages in host defense (Assuncaoet al., 2010).BCL6 gene ispro-inflammatory cytokine up regulated with 30.03 fold change in this study. This gene stimulates innate immunity against microbial infections and produced in inflammatory sites mediating the regulation of macrophage functions(Lo et al., 2013).

The mRNA of CXCL9 gene expressed with 28.63fold change after HPV infection. Other study revealed more upregulation (79 fold change) (AL-Ghazal , 2010). This increasing reflexes its important functions through stimulation of immune response and recruits T-cells to inflammatory sites (Lau and Peiris, 2009). The next up regulated gene in this study was Intrleukine-10 receptor alpha (IL10RA) with 27.59 Fold change which came in agreement with other study (24 fold change)(Baumgarthet al., 2004). IL10RA play key role in stimulation of high level of IL10 expression in monocytes, B-cells, large granular lymphocytes and T-cells. It has been shown to mediate the immunosuppressive signal of interleukin 10, and thus inhibits the synthesis of proinflammatory cytokines(Huangeet al., 2009). The expression of IL17C plays key role in innate immunity, this gene wasupregulated with 26.52 fold change. It produces peptides act as proinflammatory molecules against microbes through stimulation of NF-kappa-B and MAPK pathways. Other study also revealed increasing in IL17C level during microbial infections (Pfeifer et al., 2013). The mRNA of Interferon alpha 2 (IFNA2) gene up regulated with 24.50 fold change whereas in previous studies showed lower production of IFNA2 represented by 17.3 fold change (AL-Ghazal 2012) and 12.67 fold change (Ubolet al., 2008). IFNA2 induces hundreds of IFN stimulating genes (ISGs) which activate antiviral state in the infected cells. This protein also has been shown to be effective in reducing the symptoms and duration of the viral infections (Haoet al., 2013)

Conclusion

In conclusion the human immune system has impact factor on HPV through the up regulation of 25 key inflammatory genes.Eleven of these genes were highly produced compared with others. The immune-inflammatory gene expression profile for human patients affected with HPV was: 25 up regulated genes with mean of upregulation ranged between 6.47fold change for CCL18and 79.22 for IL1RN.

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