BIOINFORMATICS ANALYSIS OF DIFFERENTIAL GENE EXPRESSIONS IN ALCOHOLIC HEPATITIS

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Abstract:
Alcohol usage can lead to liver inflammation, which is known as alcohol hepatitis. Excessive alcohol use overloaded the liver with toxins, affecting the tissues. This study's objective is to determine the gene candidates for alcohol hepatitis in liver disease. The expression of gene profile GSE171809 was retrieved from GEO database. The GEO2R online tool was utilized to find DEGs. The KEGG pathway analysis and GO term enrichment analysis were conducted. The PPI network was framed for DEGs by utilizing the Cytoscape software to identify gene candidates and important key role pathways. A total number of 990 DEGs containing 350 upregulated DEGs and 650 downregulated DEGs were filtered with GEO2R. As per GO analysis DEGs were considerably enriched in protein DNA damage response, mitotic G2/M transition checkpoint, aminoacyl-tRNA synthetase multienzyme complex, protein-DNA complex, catalytic activity, acting on a tRNA, lysophospholipid acyltransferase activity, tRNA methyltransferase activity. For the meantime, according to KEGG pathway analysis, DEGs were significantly enriched in the Alcoholism, Glycosaminoglycan degradation, mTOR signaling pathway, p53 signaling pathway, Hippo signaling pathway - multiple species, NFkappa B signaling pathway, VEGF signaling pathway, JAK-STAT signaling pathway. Based on PPI network analysis six gene candidates were identified (AARS1, ACP1, ALDH18A1, CA6, CBX5 and SSBP1). In conclusion Our results provide a several bioinformatics study of genes, based on pathways, the genes ACP1, ALDH2, PTK2, SFN, EDA and OSM having the possibility to be targeted for liver disease.

Key words: Alcoholic hepatitis, Differentially expressed genes, Protein-protein interaction, Gene ontology, Kyoto encyclopedia of genes and genomes.

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1. Introduction

Alcoholic liver disease (ALD) is the most common type of chronic liver disease in worldwide [1]. Alcoholic fatty liver (AFL), which can be caused by hepatic inflammation, may progress to alcoholic steatohepatitis (ASH) in ALD [2]. Chronic alcoholic steatohepatitis can progress to fibrosis, cirrhosis, and, in certain cases, hepatocellular carcinoma (HCC) [3]. Furthermore, chronic (either with or without fibrosis) can result in alcoholic hepatitis, an acute symptom of alcoholic liver disease connected with liver damage and significant mortality [4].

Alcoholic hepatitis (AH) is an acute disorders caused by alcoholic liver inflammation. It can be identified by the rapid development of jaundice, malaise, painful hepatomegaly, and small systemic inflammatory properties [5]. This activity analyzes the diagnosis and treatment of alcoholic hepatitis and displays the importance of the team of experts in identifying and handling the disease [6]. Consuming excessive amounts of alcohol can cause fatty liver disease, alcoholic hepatitis, and, ultimately, cirrhosis [7].

Alcoholic liver disorders, particularly alcoholic hepatitis, is a significant cause of mortality and morbidity in the liver [8]. Alcohol and its physiological metabolites, such as acetaldehyde, can are responsible for liver damage, in alcoholic hepatitis, damage to the liver induces inflammation *via* the traditional "sterile necrosis" mechanism [9]. Increased permeability in the gut and microbiota modifications also lead to alcoholic liver damage, novel pathophysiology-based therapeutics are currently tested in patients [10].

Hepatitis is a disease of the liver which leads to cell destruction and apoptosis [11]. Most research has revealed genetic variations of alcoholic liver disease which can be utilized as non-invasive biomarkers through genome-wide association research and prospective gene analyses [12, 13, 14]. There is a critical need to discover biomarkers that are not invasive for early detection and treatment of alcoholic liver disease [15].

In this gene expression analysis based on microarray tools to use for gene expression and detecting specific DNA sequences. When the appearance levels are significantly different, the corresponding genes are called Differentially Expressed Genes (DEGs). When analyzing cDNA microarrays, univariate methods like Statistical Assessment of Linear Models for Microarray Data (LIMMA) or Microarrays (SAM) or are typically utilized to identify DEG and differential gene expression analysis based on the negative binomial distribution (DESeq) [16].

Several studies using gene expression profiling have discovered numerous differentially expressed genes (DEGs) associated with various signalling networks, biological processes, and molecular activities, which played a significant role in the existence and progression of diseases and could be utilized as a diagnostic marker and possible molecular target [17, 18,19].

The GSE171809 dataset was retrieved from the Gene Expression Omnibus (GEO) database for the current study to find DEGs between alcoholic hepatitis and normal samples. Consequently, performing enrichment analysis on gene sets is one of the primary applications of GO term enrichment research. An enrichment analysis, for instance, can be used to determine what GO terms are under-represented (or over-represented) utilizing annotations for a gene set that is up-regulated during specific circumstances, and PPI (protein-protein interaction) network analysis were performed to discover candidate genes as alcoholic hepatitis in liver disease therapeutic targets and biomarkers worthy of further progress.

2.Materials and methods

2.1. Microarray Data sets: The data set based on the GPL18573 Illumina Next Seq 500 (Homo sapiens) was retrieved from GEO (https://www.ncbi.nlm.nih.gov/gds/?term=GSE171809). Total of 11 samples were analysed, 3 of normal samples, and 8 alcoholic hepatitis [20].

2.2. DEG analysis:

GEO2R can be used to compare two or more sample groups and find genes that express differentially exp ressed in various conditions during studies [21]. The sample's gene expression profiles from subjects with alcoholic hepatitis in the dataset GSE171809 were as sociated to recognize DEGs. This analysis was exam ined by utilizing GEO2R (https://www.ncbi.nlm.nih. gov/geo/geo2r/?acc=GSE171809) through R progra mming based analysis of the microarray data [22]. l og (fold change) $|\geq 1$ and $P<0.05$ were the cut-off crit eria. A heat map of these DEGs was drawn using R Studio programmer 4.3.1. (The R Foundation for Sta tistical Computing Platform: x86_64-w64-mingw32/ x64).

2.3. Functional and pathway enrichment analysis

Pathway enrichment studies incorporate statistical processes which organize the results of pathways by frequency in the gene list and express their enrichment as a probability value known as the p-value. Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) is to perform enrichment analysis on gene sets, an enrichment analysis will determine which GO terms are over-represented utilizing annotations for specific set of genes that are up-regulated under certain conditions, the DEGs were enriched utilizing the Database of Annotation Visualization and Integrated Discovery (DAVID 6.8; https://david.ncifcrf.gov/) [23]. Biological process (BP), molecular function (MF), and cellular component (CC) were the GO categories, a statistically significant difference was defined as $P<0.05$.

2.4. PPI (Protein-Protein Interaction) analysis:

String DB (https://string-db.org/) it is used for creating a PPI network, this website provides predicted and validated protein interactions [24]. The combined analysis used the number of nodes:178, number of edges:284, average node degree:3.19, avg. local clustering coefficient:0.394, expected number of edges:183, and PPI enrichment p-value:3.9e-12.

The screened PPI network was imported into Cytoscape 3.2.1 (http://www.cytoscape.org/) to recognize censorious gene modules and hub genes [25]. Nodes with a high degree (≥2 fold the median number of associates with other nodes) were regarded as important nodes and higher degree nodes (≥5 fold the median number of connections with other nodes) were considered as hub nodes.

3. Results

3.1. Identification of DEGs: Based on the previously mentioned threshold log (fold change) ≥1 and $P<0.05$, a total of 990 DEGs including 350 upregulated DEGs and 650 downregulated DEGs were filtered with GEO2R (Figure.1).

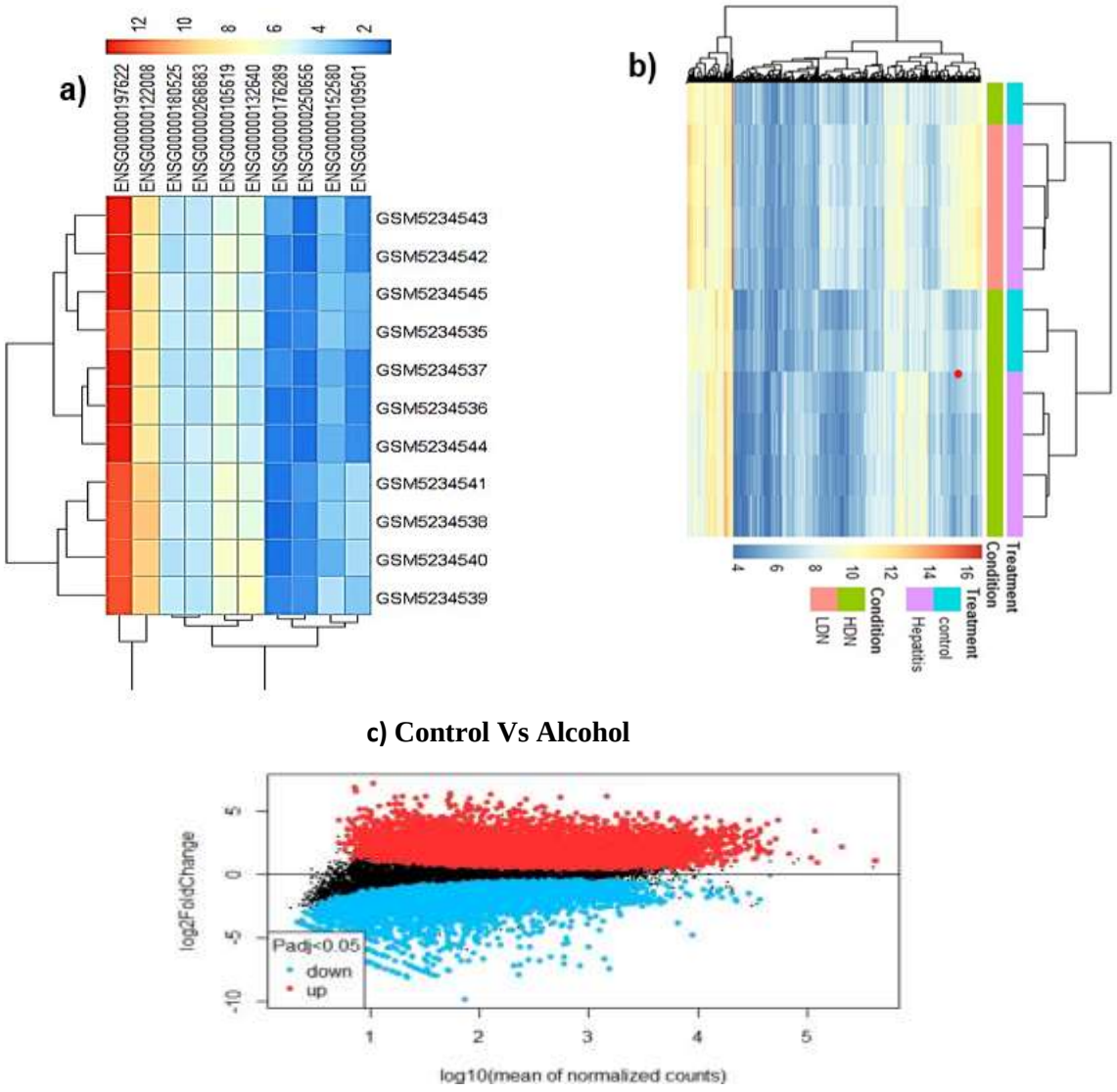


Figure.1 a, b) Heat-map of the 20 top downregulated and upregulated gens. **c).** DEGs in alcoholic hepatitis which represent control, and alcoholic hepatitis ($P<0.05$).

3.2. GO analysis and KEGG Pathway:

In the GO category BP, upregulated genes were mostly enriched, mainly associated with like Mitotic G2/M transition checkpoint, Phagocytosis, Fc-gamma receptor signalling pathway, Ribonucleoprotein complex biogenesis, Negative regulation of axonogenesis and Alpha-amino acid biosynthetic process whereas downregulated genes were mainly associated with rRNA-containing ribonucleoprotein complex export from nucleus, Production of molecular mediator of immune response, Protein export from nucleus Fc receptor signalling pathway, Protein-containing complex localization and Purine-containing compound metabolic process (Figure. 2a) (Table.1). In the GO category MF, the genes upregulated were mostly associated in Antigen binding, Immunoglobulin receptor binding, translation regulator activity and MHC protein complex binding (Figure. 2b) (Table.1), similarly, downregulated genes were mostly associated in mRNA 5'-UTR binding, Lyase activity, Lysophospholipid acyltransferase activity and Carboxylic ester hydrolase activity **(Table.1)**. Lastly, regarding the GO category CC, the upregulated genes were mostly associated in the Immunoglobulin complex, Eukaryotic 48S preinitiation complex, blood microparticle and Host cellular component (Figure.2c) (Table.1), whereas downregulated genes were mostly associated in Organelle ribosome, Mitochondrial matrix, Kinetochore and Lamellipodium **(Table.1).**

Table. 1. Go analysis of down and up regulated genes in alcoholic hepatitis (P<0.05).

Biological process (BP)					
Upregulated genes					
Sl.No	**ID**	**Description**	**P value**	**Genes**	**Count**
1.	GO:0006909	Phagocytosis	0.0143	PTK2, IGHA-2, CLN3, IGKV2-28, XKR8, IGHV3-15, IGKV139, IGKV1-17, IGKV1-5, IGKC, IGHV1-3, IGHM/IGLV1-47, IGHV3-74, IGLV3-19, IGHV3-23.	16
2.	GO:0038094	Fc-gamma receptor signaling pathway	0.0784	PTK2, IGKV2-28, IGKV1-39, IGKV1-17, IGKV1-5, IGKC, IGLV1-47, IGLV3-19, IGHV3-23.	9
3.	GO:0022613	Ribonucleoprotein complex biogenesis	0.0950	NUP88, METTL5, CLNS1A, EIF3B, EIF3L, RPL7L1, PA2G4, EIF3H, RIOK2, DDX1, RBIS, UTP3, RAN, SDAD1, EBNA1BP2, RBFA.	16
4.	GO:0050771	Negative regulation of axonogenesis	0.0935	IFRD1, PTK2, TRAK2, PTPRO.	4
5	GO:1901607	Alpha-amino acid biosynthetic process	0.0230	CLN3, ALDH18A1. SHMT2.	3
6	GO:0044818	Mitotic G2/M transition checkpoint	0.0350	NAE1, TRIM39.	2
Down regulated genes					
1.	GO:0071428	rRNA-containing ribonucleoprotein complex export from nucleus	0.08581	NUP88, RIOK2, RAN, SDAD1.	4
2.	GO:0002440	Production of molecular mediator of immune response	0.0784	STX4, TMIGD2, IGKV2-28, IGKV1-39, IGKV1-17, IGKV1-5, IGKC, DDX1, IGLV1-47, IGLV3-19, IL18R1, XBP1.	12
3.	GO:0006611	Protein export from nucleus	0.0950	NUP88, SEH1L, SFN, RIOK2, RAN, SDAD1, NUP43, XPO5, XPO6.	9
4.	GO:0038093	Fc receptor signalling pathway	0.0410	PTK2, IGKV2-28, IGKV1-39, IGKV1-17, IGKV1-5, IGKC, IGLV1-47, IGLV3-19,	9

				IGHV3-23.	
5	GO:0031503	Protein-containing complex localization	0.0230	DBN1, NUP88, SEH1L, RIOK2, RAN,SDAD1, NUP43.	7
6	GO:0072521	Purine-containing compound metabolic process	0.0450	FHIT, NUP88, SEH1L, PAICS, HACD2, ATP5F1A, NUDT9, NME1, RAN, NUP43, SHMT2.	11

Molecular Function (MF)

Upregulated genes

Sl.No	ID	Description	P value	Genes	Count
1.	GO:0003823	Antigen binding	0.01834	IGHA2, IGHV3-15. IGKV1-39, IGKV1-17, IGKV1-5, IGKC, IGHV1-3, IGHM, IGLV1-47, IGHV3-74, IGLV3-19, IGHV3-23.	12
2.	GO:0034987	Immunoglobulin receptor binding	0.0193	IGHA2, IGHV3-15, IGKC, IGHV1-3, IGHM, IGHV3-74, IGHV3-23.	7
3.	GO:0045182	translation regulator activity	0.0958	EIF3B, EIF3L, EIF3H, AARS1, SHMT2.	5
4.	GO:0023023	MHC protein complex binding	0.0234	KLRC1, MS4A1	2

Down regulated genes

1.	GO:0048027	mRNA 5'-UTR binding	0.0699	RPS13, RPS3A, NCL, SHMT2.	4
2.	GO:0016829	Lyase activity	0.0758	CA6, PAICS, HACD2, UROSCA4, TSEN34, SHMT2.	7
3.	GO:0071617	Lysophospholipid acyltransferase activity	0.0138	ABHD4, AGPAT5.	2
4.	GO:0052689	Carboxylic ester hydrolase activity	0.0481	ABHD4, CES4A, AARS1, IARS1	4

Cellular Component (CC)

Upregulated genes

Sl.No	ID	Description	P value	Genes	Count
1.	GO:0019814	Immunoglobulin complex	0.0116	IGHA2, IGKJ1, IGKV2-28, IGHV3-15, IGKV1-39, IGKV1-17, IGKV1-5, IGKC, IGHV1-3, IGHM, IGLV1-47, IGHV3-74, IGLV3-19, IGHV3-23.	14
2.	GO:0033290	Eukaryotic 48S preinitiation complex	0.0316	EIF3B, EIF3L, EIF3H.	3
3.	GO:0072562	blood microparticle	0.0475	IGHA2, IGKV1-39, IGKV1-17, IGKV1-5, IGKC, IGHM, IGLV1-47, IGHV3-23.	8
4.	GO:0018995	Host cellular component	0.0598	NUP88, SEH1L, RAN, NUP43.	4

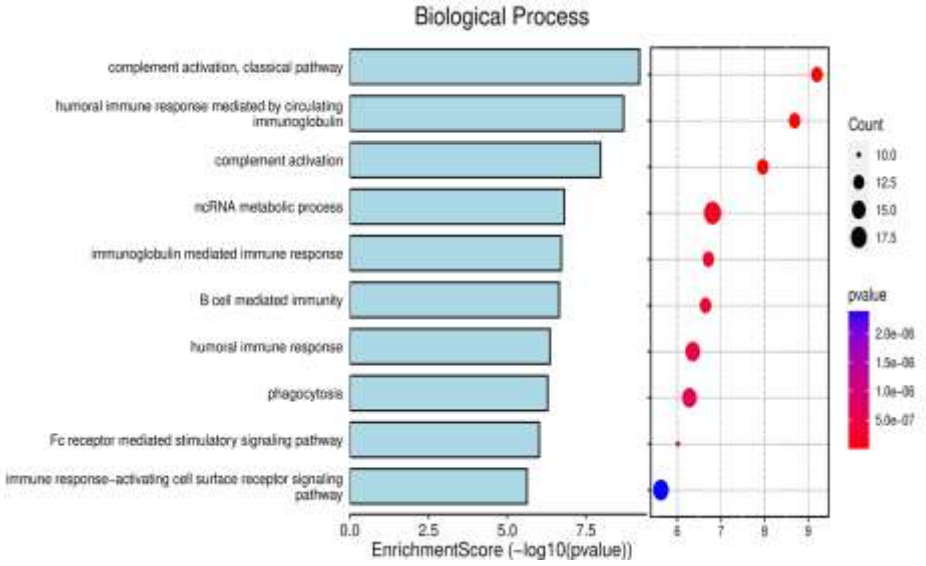
Down regulated genes

1.	GO:0005759	Organellar ribosome	0.0837	MRPL42, MRPS35, MRPS9, MRPS16.	4
2.	GO:0005759	Mitochondrial matrix	0.0303	MRPL42, IDH3A, ATP5F1A, MRPS35, NUDT9, SSBP1, MRPS9, MRPS16, SHMT2.	9

3.	GO:0000776	Kinetochore	0.0373	SEH1L, CBX5, NUP43, PHF6.	4
4.	GO:0030027	Lamellipodium	0.0410	PTK2, IGKV2-28, IGKV1-39, IGKV1-17, IGKV1-5, IGKC, IGLV1-47, IGLV3-19, IGHV3-23.	9

PTK2:PTK2 Protein Tyrosine Kinase 2, NME1: Nucleoside Diphosphate Kinase 1, CLN3: Lysosomal/Endosomal Transmembrane Protein, IGHA2: Immunoglobulin Heavy Constant Alpha 2, PHF6: PHD Finger Protein 6, IGKJ1: Immunoglobulin Kappa Joining 1, SSBP1: Single Stranded DNA Binding Protein 1, CBX5: Chromobox 5, IDH3A: Isocitrate Dehydrogenase (NAD (+)) 3 Catalytic Subunit Alpha, MRPS9: Mitochondrial Ribosomal Protein S9, AGPAT5: 1-Acylglycerol-3-Phosphate O-Acyltransferase 5, MRPS16: Mitochondrial Ribosomal Protein S16, IGKV2-28: Immunoglobulin Kappa Variable 2-28, CES4A: Carboxylesterase 4A, MRPS35: Mitochondrial Ribosomal Protein S35, EIF3H: Eukaryotic Translation Initiation Factor 3 Subunit H, ATP5F1A: ATP Synthase F1 Subunit Alpha, HACD2: 3-Hydroxyacyl-CoA Dehydratase 2, PAICS: Phospho ribosyl amino imidazole succino carboxamide Synthase, AARS1: Alanyl-TRNA Synthetase 1, RPS3A: Ribosomal Protein S3A, XKR8: XK Related 8, NCL: Nucleolin, ABHD4: Abhydrolase Domain Containing 4, N-Acyl Phospholipase B, TSEN34: TRNA Splicing Endonuclease Subunit 34, IARS1: Isoleucyl-TRNA Synthetase 1, CA6: Carbonic Anhydrase 6, MRPL42: Mitochondrial Ribosomal Protein L42, IGHV3-15: Immunoglobulin Heavy Variable 3-15, IGKV139: Immunoglobulin Kappa Variable 1-39, RPS13: Ribosomal Protein S13, IGKV1-17: Immunoglobulin Kappa Variable 1-17, IGKV1-5: Immunoglobulin Kappa Variable 1-5, IGHV1-3: Immunoglobulin Heavy Variable 1-3, IGHM: Immunoglobulin Heavy Constant Mu, IGLV1-47: Immunoglobulin Lambda Variable 1-47, IGHV3-74: Immunoglobulin Heavy Variable 3-74, IGLV3-19: Immunoglobulin Lambda Variable 3-19, IGHV3-23: Immunoglobulin Heavy Variable 3-23, NUP88: Nucleoporin 88, METTL5: Methyltransferase 5, N6-Adenosine, CLNS1A: Chloride Nucleotide-Sensitive Channel 1A, EIF3B: Eukaryotic Translation Initiation Factor 3 Subunit B, KLRC1: Killer Cell Lectin Like Receptor C1, NUDT9: Nudix Hydrolase 9, EIF3L: Eukaryotic Translation Initiation Factor 3 Subunit L, RPL7L1: Ribosomal Protein L7 Like 1, PA2G4: Proliferation-Associated 2G4, MS4A1: Membrane Spanning 4-Domains A1, EIF3H: Eukaryotic Translation Initiation Factor 3 Subunit H, RIOK2: RIO Kinase 2, IFRD1: Interferon Related Developmental Regulator 1, SHMT2: Serine Hydroxymethyltransferase 2, TRIM39: Tripartite Motif Containing 39, RAN: RAN, Member RAS Oncogene Family, SDAD1: SDA1 Domain Containing 1, STX4: Syntaxin 4, TMIGD2: Transmembrane and Immunoglobulin Domain Containing 2, IGKV1-39: Immunoglobulin Kappa Variable 1-39, IGKC: Immunoglobulin Kappa Constant, DDX1: DEAD-Box Helicase 1, IL18R1: Interleukin 18 Receptor 1, XBP1: X-Box Binding Protein 1, SEH1L: SEH1 Like Nucleoporin, SFN: Stratifin, NUP43: Nucleoporin 43, XPO5: Exportin 5, XPO6: Exportin 6, DBN1: Drebrin 1, FHIT: Fragile Histidine Triad Diadenosine Triphosphate.

a)



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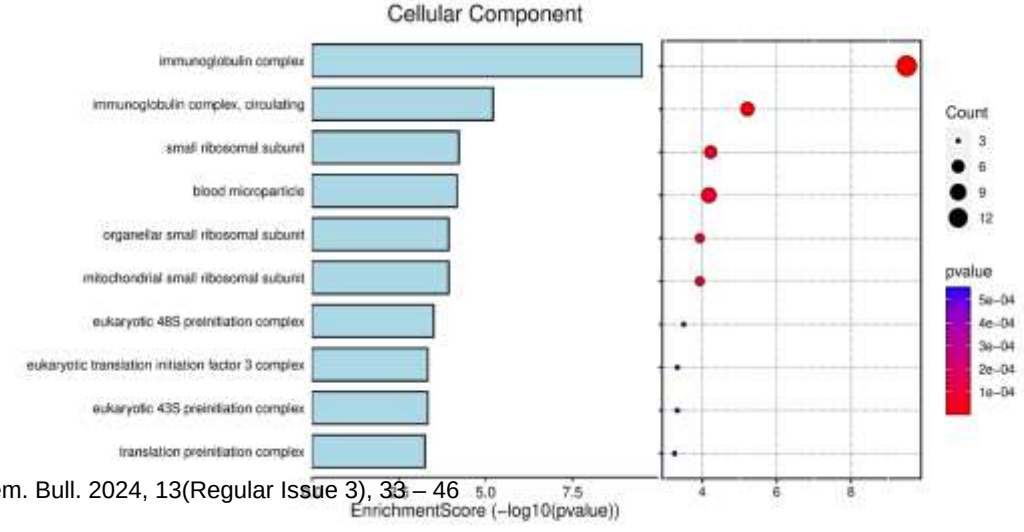

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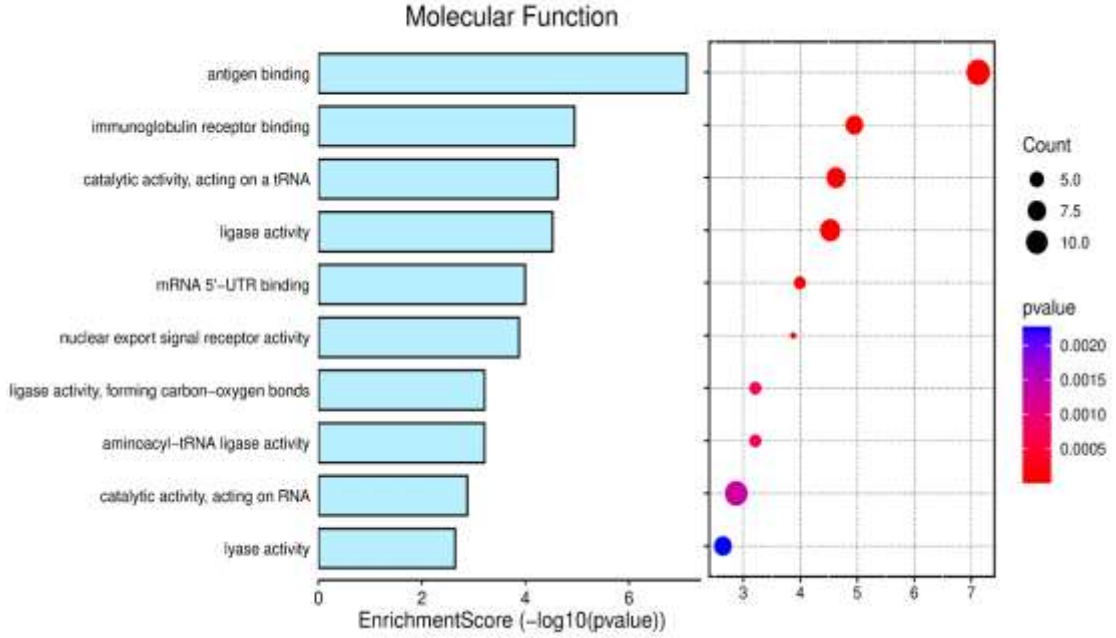

Figure 2: Enriched Gene Ontology analysis of differentially expressed genes; **2a)** BP: biological process; **2b**) CC: cellular component; **2c)** MF: molecular function.

The pathway analysis conducted by KEGG showed that DEGs were significant association with the Hippo signaling pathway - multiple species, Alcoholism, Glycosaminoglycan degradation, mTOR signaling pathway, p53 signaling pathway, VEGF signaling pathway, NF-kappa B signaling pathway, JAK-STAT signaling pathway. Aminoacyl-tRNA biosynthesis, Nucleocytoplasmic transport, Biosynthesis of amino acids, Nitrogen metabolism, Biosynthesis of cofactors, Mismatch repair, Purine metabolism, Systemic lupus erythematosus, Pentose and glucuronate interconversion and DNA replication (**Figure3).**

Table 2. Analysis of up- and downregulated genes in alcoholic hepatitis is provided in the Kyoto Encyclopedia pathway of Genes and Genomes (P<0.05).

KEGG Pathway					
Down regulated genes					
Sl.No	**ID**	**Description**	**P value**	**Genes**	**Count**
1.	hsa00970	Aminoacyl-tRNA biosynthesis	0.0311	EPRS1, AARS1, FARSB, IARS1, QRSL1.	4
2.	hsa03013	Nucleocytoplasmic transport	0.0320	NUP88, SEH1L, RAN, NUP43, XPO5, XPO6.	6
3.	hsa01230	Biosynthesis of amino acids	0.0523	IDH3A, RPE, ALDH18A1, SHMT2.	4
Up regulated genes					
1.	hsa01240	Biosynthesis of cofactors	0.0363	AKR1A1, UROS, EPRS1, NME1, SHMT2.	5

2.	hsa00230	Purine metabolism	0.05730	FHIT, PAICS, NUDT9, NME1.	4
3.	hsa05322	Systemic lupus erythematosus	0.0450	H2BC18, H2BC21, H2AC18, H2BC4.	4
4.	hsa03030	DNA replication	0.0610	SSBP1, POLD2	2

FARSB: Phenylalanyl-TRNA Synthetase Subunit Beta, EPRS1: Glutamyl-Prolyl-TRNA Synthetase 1, QRSL1: Glutaminyl-TRNA Amidotransferase Subunit QRSL1, XPO5: Exportin 5, AKR1A1: Aldo-Keto Reductase, Family 1 Member A1, UROS: Uroporphyrinogen III Synthase, H2BC18: H2B Clustered Histone 18, H2BC21: H2B Clustered Histone 21, H2AC18: H2A Clustered Histone 18, H2BC4: H2B Clustered Histone 4, POLD2: DNA Polymerase Delta 2, Accessory Subunit.

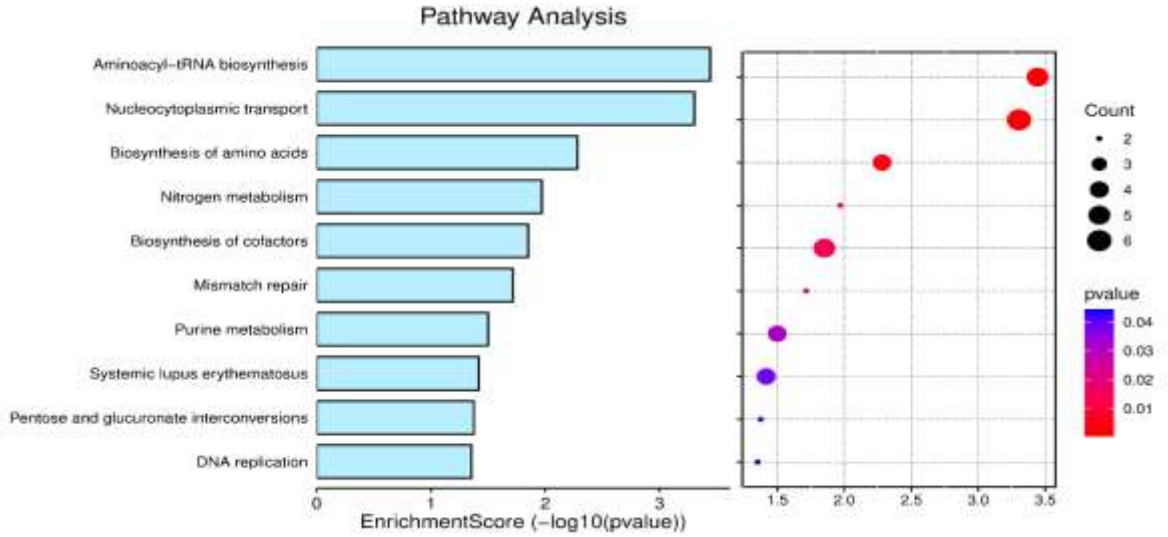

Figure.3. The pathway of KEEG analysis of upregulated and down regulation genes in alcoholic hepatitis (P<0.05).

3.3. PPI analysis:

The PPI network analysis results as available a total of 20 genes were considered as number for hub genes (Figure. 4) (Table.3). Finally, as presented in (Table.4) (Figure.5), six hub genes involved in these alanyl-TRNA synthetase 1 (AARS1) with number of nodes 11 and number of edges 55, acid phosphatase 1 (ACP1), with number of nodes 11 and number of edges 17 aldehyde dehydrogenase 18 family member A1 (ALDH18A1) number of nodes 11 and number of edges 44, carbonic anhydrase 6 (CA6) number of nodes 11 and number of edges 17, chromobox 5 (CBX5) number of nodes 11 and number of edges 54, single stranded DNA binding protein 1 (SSBP1) number of nodes 11 and number of edges 24.

Table: 3. The hub node gene analysis of differentially expressed genes.

Hub node 1	Hub node 2	Node 1 accession	Node 2 accession	score
PTK2	NF2	ENSP00000299300	ENSP00000248437	0.685
SSBP1	ATP5F1A	ENSP00000299300	ENSP00000381736	0.439
TUBA4A	CCT2	ENSP00000209875	ENSP00000358151	0.567

NF2	PTK2	ENSP00000366654	ENSP00000300900	0.569
H2BC21	H2BC18	ENSP00000358151	ENSP00000445831	0.909
H2BC21	CBX5	ENSP00000358151	ENSP00000209875	0.567
H2BC18	H2BC21	ENSP00000445831	ENSP00000358151	0.989
CCT2	TUBA4A	ENSP00000341189	ENSP00000344666	0.685
CCT2	ATP5F1A	ENSP00000419665	ENSP00000381736	0.503
CBX5	H2BC21	ENSP00000248437	ENSP00000299300	0.578
CA6	CA4	ENSP00000344666	ENSP00000341189	0.578
CA4	CA6	ENSP00000300900	ENSP00000366654	0.569
ATP5F1A	SSBP1	ENSP00000381736	ENSP00000419665	0.503
ATP5F1A	CCT2	ENSP00000381736	ENSP00000299300	0.439
ATP5F1A	ALDH18A1	ENSP00000381736	ENSP00000360268	0.620
ALDH18A1	ATP5F1A	ENSP00000360268	ENSP00000381736	0.620

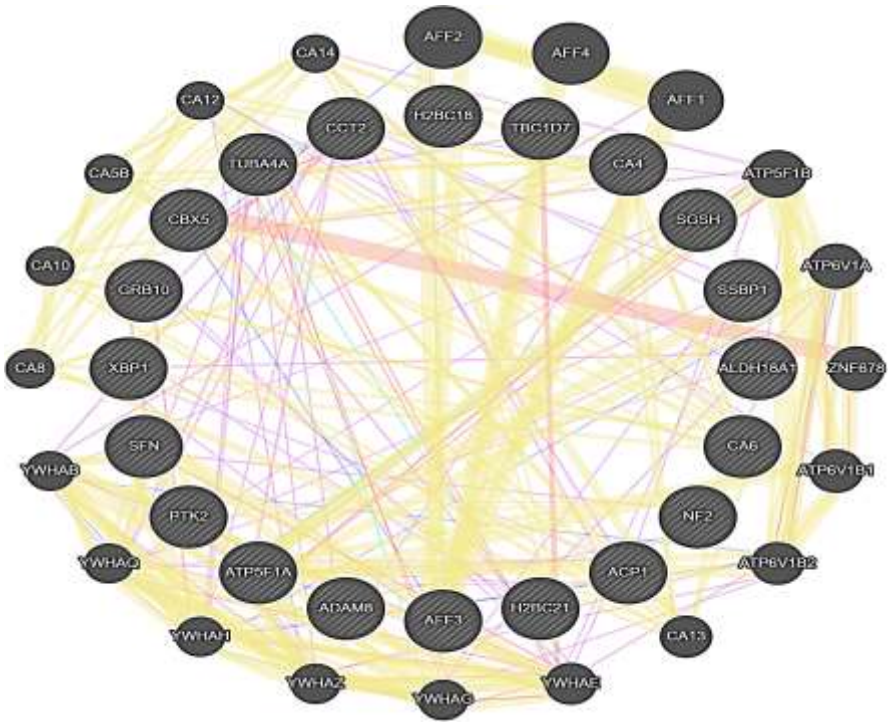

Figure. 4. Genes with differentially expressed in core protein-protein interaction network with number of nodes:20, number of edges:8, average node degree:0.8, avg. local clustering coefficient:0.45, PPI enrichment p-value:0.39.

Table.5. The number of genes selected from protein-protein interaction analysis**.**

Hub Genes	Number of nodes	Number of edges	Average node degree	Average local clustering coefficient	Expected number of edges	PPI enrichment p-value
AARS1	11	55	10	1	12	< 1.0e-16
ACP1	11	17	3.09	0.847	10	0.0389
ALDH18A1	11	44	8	0.854	11	6.79E-14
CA6	11	17	3.09	0.893	10	0.0331
CBX5	11	54	9.82	0.982	24	7.54E-08
SSBP1	11	24	4.36	0.937	11	0.000589

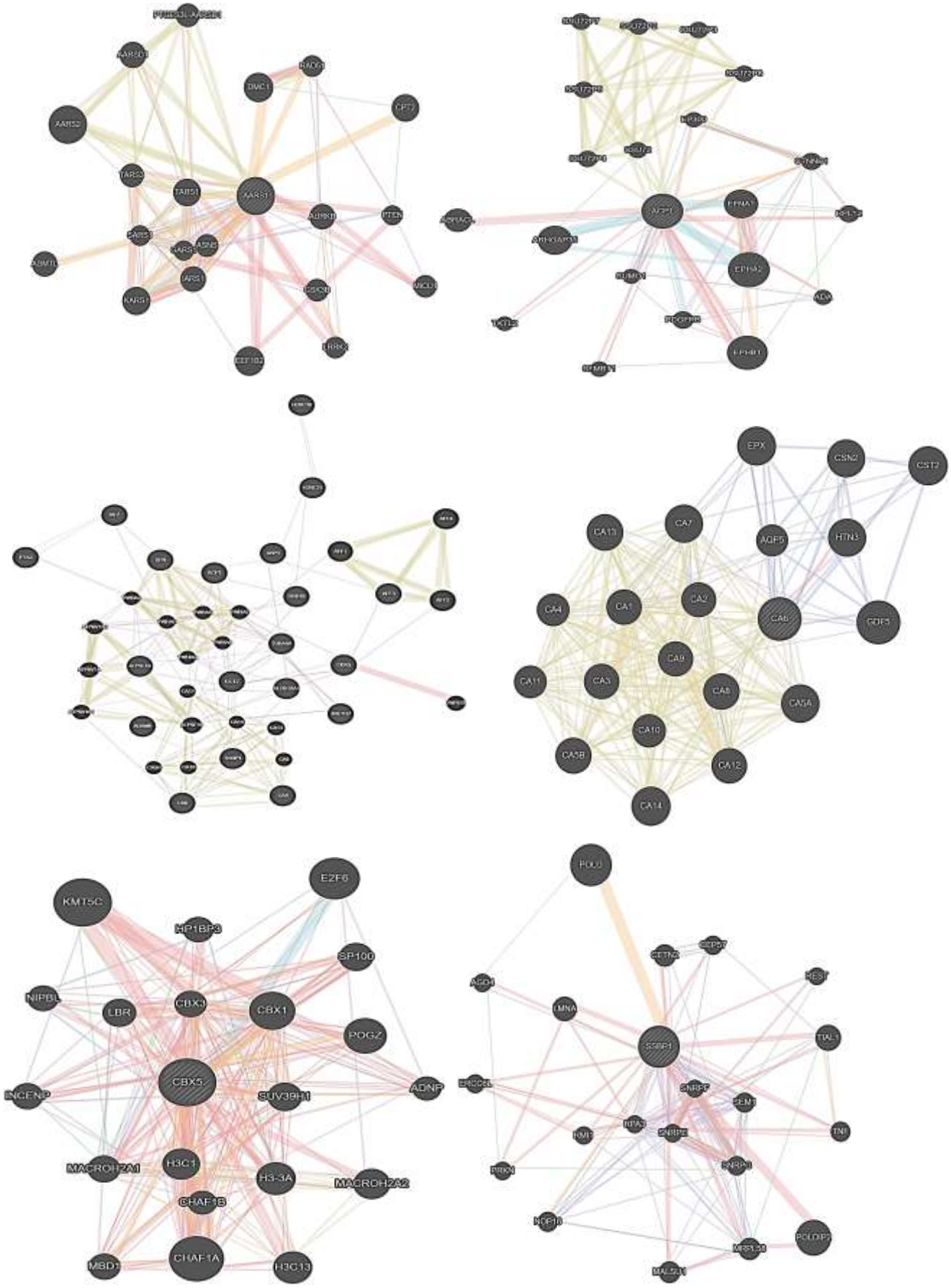

Figure. 5. The PPI (protein-protein interaction) conducted for AARS1, ACP1, ALDH18A1, CA6, CBX5 and SSBP1 by STRING analysis.

4. Discussion

Alcohol-related liver disease (ALD) is the term used to describe liver damage carried due to a high alcohol intake [26]. Alcoholic hepatitis, alcoholic cirrhosis, and alcoholic fatty liver, or steatosis, are the stages of alcoholic liver disease progression [27]. In the earliest stages of alcoholic liver disease, severe acute hepatitis (AH) is an incurable condition with a very high 180-day mortality rate [28].

In this study, we recognized a total of 990 substantial DEGs among alcoholic hepatitis and normal samples, including 350 upregulated genes and 650 downregulated genes, and examined a sequence of bioinformatics study to screen the number of genes and pathways associated to alcoholic hepatitis in liver disease.

DEGs were examined in both KEGG pathway analysis and GO term enrichment analysis for functional annotation. As the result of GO term enrichment analysis, DEGs might act significant parts in alcoholic hepatitis in liver disease, through protein-DNA complex subunit organization, phagocytosis, Fc receptor mediated stimulatory signaling pathway, rRNA-containing ribonucleoprotein complex export from nucleus protein export from nucleus, DNA damage response, mitotic G2/M transition checkpoint, cellular amino acid biosynthetic process, translation preinitiation complex, aminoacyl-tRNA synthetase multienzyme complex, protein-DNA complex, catalytic activity, acting on a tRNA, lysophospholipid acyltransferase activity, tRNA methyltransferase activity. For the meantime,

KEGG pathway analysis revealed that DEGs were mainly enriched in the Alcoholism, Glycosaminoglycan degradation, mTOR signaling pathway, Hippo signaling pathway - multiple species, VEGF signaling pathway, p53 signaling pathway, NF-kappa B signaling pathway, JAK-STAT signaling pathway, Aminoacyl-tRNA biosynthesis, Nucleocytoplasmic transport, Biosynthesis of amino acids, Nitrogen metabolism, Biosynthesis of cofactors, Mismatch repair, Purine metabolism, Systemic lupus erythematosus, Pentose and glucuronate interconversion and DNA replication.

Moreover, by construction of the protein-protein interaction, 6 genes were recognized, which applied an important consequence on the alcoholic hepatitis in liver disease. The selected genes of AARS1, ACP1, ALDH18A1, CA6, CBX5 and SSBP1. alanyl-tRNA synthetase-1 (AARS1), as per previous studies also conformed the gene may have associated with recurrent alcoholic hepatitis liver disease [29]. Acid Phosphatase 1 (ACP1) Gene Ontology (GO) annotations related to this gene include phosphatase activity and acid phosphatase activity. Acetaldehyde dehydrogenase 2 (ALDH2) is the key enzyme responsible for metabolism of the alcohol metabolite acetaldehyde in the liver.

As per KEGG pathway analysis VEGF signaling pathway the candidate gene (protein tyrosine kinase 2) PTK2 mention in earlier studies overexpression of circ PTK2 suppresses *via* the miR-200c/SIK2/PI3K/Akt axis, PTK2 inhibits the progression of nonalcoholic fatty liver disease [30]. p53 signaling pathway the candidate gene sulforaphane the study has explained SFN ameliorates lipid metabolism disorders in NAFLD mice by upregulating FGF21/FGFR1 pathway and may become a promising intervention to treat or relieve non-alcoholic fatty liver disease [31]. NF-kappa B signaling pathway the candidate gene Ectodysplasin A (EDA) *in vivo* research have revealed EDA is a protein secreted by the liver that causes skeletal muscle insulin resistance. It has been found to be elevated in the liver and plasma of obese mice. [32]. JAK-STAT signaling pathway the candidate gene Oncostatin M (OSM) is a pleiotropic cytokine of the interleukin (IL)-6 family

that contributes to the development of chronic liver disease [33]. The analysis of KEGG pathway and DEGs were mainly enriched the genes are associated in alcoholic hepatitis in liver disease.

5.Conclusion

Our examination attempted to recognized a few candidate genes and using a series of bioinformatics analyses on DEGs between alcoholic hepatitis sample datasets and normal samples datasets, a pathway regulatory network nearly associated to liver disease was identified. Based on PPI network analysis six gene candidates were identified (AARS1, ACP1, ALDH18A1, CA6, CBX5 and SSBP1). DEGs and KEGG pathway analysis revealed such as ACP1, ALDH2, PTK2, SFN, EDA and OSM having the possibility to be targeted for the diagnosis and treatment of alcoholic hepatitis liver disease. Moreover, the limitation of clinical validation of this study, the obtained or prediction results through bioinformatics analysis can be confirmed by additional experimental studies such as Western blot and qRT-PCR.

6. Conflict of Interest statement

None

7. Acknowledgements

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