Retinoblastoma (RB) is the most common malignant tumor of the eye in children. Inactivation of both copies of the RB1 gene in retina is known to be the cause of cancer. Here, we present metabolomic studies on vitreous humor samples to identify differential metabolites in RB patients that can provide a direct or indirect link to the pathways found in cancerous tissue. 9 patient and 2 controls samples were used. The extracted samples were subjected to LC/TOF-MS and GC/TOF-MS analysis. More than 1000 features were identified using these two techniques. Wide variety of compounds ranging from amino acids, carbohydrates, nucleobases and lipids were identified. Among lipids, Phosphatidyl cholines (PC), ether linked phosphatidyl ethanolamines (PE), ceramides, sphingomyelins and sphinganines were identified. Lipids, especially PGs and other linked PEs were found to be up regulated in patient samples. Many of the other lipids found to be 5 folds more in patient samples. Carnitines and free fatty acids were also up regulated in patient samples. As the biosynthesis of other lipids starts in peroxisomes, this study suggests an altered peroxisomal metabolism in these patients.

**Experimental**

**Method**

The samples from 9 patient and 2 controls were extracted using methanol: ethanol (1:1 v/v). The extracted samples were subjected to LC/TOF-MS and GC/TOF-MS analysis. For LC-TOF analysis, data was acquired using electrospray ionization in positive and negative ion modes using modified polar reverse phase C18 column and HILIC column. Molecular features were searched against MELTUN library and confirmed by MELTUN library using data dependent MS/MS acquisition. For GC-TOF analysis, data was acquired using EI source on a DB-5ms column. The results were searched against Fiehn RTL library. Accurate mass libraries along with SimLipid software facilitated analysis of various class of metabolites including lipids. Genomic and metabolomics data were co-visualized in the pathway context using the MetMS analysis tool of GeneSpring 13.1, which enabled simultaneous viewing of the differential entities from both gene expression and metabolomics. Table 2 shows the list of predominant pathways as revealed by combined analysis of LCMS of vitreous humor and gene expression.

**Results and Discussion**

A pair wise analysis between samples (9 patient and 2 controls) within vitreous humor (C18 Pos) metabolomics experiments is shown in figure 3. The 9 patient samples are classified based on clinical and pathological risk as high risk (H), low risk (L) and no risk (N). The correlation analysis followed by clustering showed the relationship between the three groups of 9 patients. The results showed that high risk group patients correlate positively with each other marked by red color. Most of the other samples showed no (yellow) correlation or negative (blue) correlation with controls.

**Conclusions**

• Vitreous humor being in closest proximity to the retinoblastoma tissue showed characteristics exo-metabolites from the cancer tissue.
• Different classes of metabolites were confirmed using LCMS and GCMS techniques.
• Accurate mass LCMS libraries along with SimLipid software facilitated analysis of various class of metabolites including lipids.
• Pathway analysis of differential metabolites using Genomic software yielded key biological pathways which were also reflected in the genomics study (data not shown).
• Up regulation of phosphatidylcholines, free fatty acids and lipid transporters like carnitines Indicates an altered lipid metabolism in the patients.
• Although retrieval of vitreous humor would require evasive procedures, a more detailed study using other biological fluids such as tears are underway.