Determining the impact of Vitamin E & Selenium supplementation on gene expression in the brains of mice infected with T. *gondii*

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1 Background

Previous studies have shown that dietary supplementation with antioxidants is harmful during murine infection with the protozoan parasite, *Toxoplasma gondii*. In both Swiss Webster and C57BL/6 mice, supplementation with vitamin E and selenium resulted in an increased number of tissue cysts, tissue pathology, and weight loss during *T. gondii* infection (McCarthy & Davis, 2003). Microarrays are used widely these days to determine the significant genes in experimental models [3]. Various methods are implemented for analyzing the microarray data. Normalization is usually required on raw intensities obtained from microarray for variance stabilization. This normalized data will further require some statistical tests for determining genes with significant difference. The overall goal of the present study was to determine the impact of Vitamin E & Selenium supplementation on gene expression in the brains of mice infected with *T. gondii* with an Microarray analysis.

2 Materials and Methods

A Data Driven Harr Fisz (DDHF) and Z score transformations were done separately on the Agilent microarray raw intensities obtained from the brains of non-infected and infected mice with *Toxoplasma gondii* parasite, which were further processed by forming patterns on fold changes for finding significant genes specific to each treatment [1, 3]. We used 4 treatments, mice with normal diet, mice with Vitamin E and Selenium supplemented diet, *T. gondii* infected mice on normal diet and *T. gondii* infected mice with Vitamin E and Selenium supplemented diet. There were 4 biologi-

cal and 3 technical replicates with 12 mice per treatment. A distribution plot was made on fold changes and cutoffs for ratios for the significant peaks were set to 1.4 for the upper ratio and 0.71 for the lower ratio. The results were scored as +1, 0, or -1. There sum gave a score matrix where down regulated genes were set to -2, non-regulated to 0 and up regulated to 2, forming patterns. A separate analysis was made by performing ANOVA with a Duncan Post hoc test and subsequent pattern formation similar to first analysis. A Statistical Analysis of Microarray (SAM) was also performed on raw intensities to validate the significant genes [2]. A comparison was made between patterns obtained from all the different analyses to find common significant genes.

3 Results and Conclusions

From the analysis, we were successful in delineating the significant processes in the mice infected with *T. gondii* parasite. Furthermore we found out the effects of vitamin E and selenium supplementation in the mice infected by the same parasite by comparing these significant genes in that particular treatment group. Immunological genes like TNF-alfa, IL-2, IL-4, INF-beta, Gama globulin's, and other inflammatory cytokine genes were significantly up regulated in *T. gondii* infected mice with Vitamin E and Selenium supplementation compared to control. A total of 55 genes relating to increased inflammation and pathology were found up regulated in *T. gondii* infected mice with Vitamin E and Selenium supplementation, which were down regulated in normal mice with Vitamin E and Selenium supplementation. The pathway analysis of these genes is presently carried out to understand their interaction's. Understanding of these genes will explore more effective treatment options for *T. gondii* parasite infestation in humans.

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References

1. Chris Cheadle, Marquis P. Vawter, William J. Freed and Kevin G. Becker, (May 2003). Journal of Molecular Diagnostics, Vol. 5 (No. 2).

2. Chu, G., Narasimhan, B., Tibshirani, R. & Tusher, V. (2002), Signicance analysis of micro-arrays (sam) software. Available: http://www-stat.stanford.edu/~tibs/SAM/ via the Internet. Accessed 2003 July 16
3. E. S. Motakis, G. P. Nason, P. Fryzlewicz and G. A. Rutter, . (2006). Variance stabilization and normalization for one-color microarray data using a data-driven multiscale approach. Bio Informatics, Vol. 22 .
4. McCarthy, S. M., C. D. Davis. (2003). Pro-oxidant diet provides protection during murine infection with Toxoplasma gondii. Journal of Parasitology 89: 886-894.
5. Wahrman. MZ, Halsey. H, Anderson. L, Taylor. J, Lipkin. M, (1987). Expression of cloned sequences in biopsies of human colonic tissue and in colonic carcinoma cells induced to differentiate in vitro. Cancer Research, Vol 47 (22): 6017-6021.