Correlation between oxidatives stress and chemokines in the cerebrospinal fluid of multiple sclerosis patients.

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BACKGROUND

Chemokines have been shown to modulate generation of reactive oxygen species (ROS) at the site of inflammation. This association between ROS and inflammation, was viewed as a one-way process, i.e. immune cells activated by chemokines produced ROS. However, excessive ROS production disturbs redox status, damages macromolecules, and can modulate the expression of a variety of immune and inflammatory molecules, exacerbating inflammation and affecting tissue damage. The aim of the current study is to establish a relationship between oxidative stress and chemokines with disease activity in MS.

OBJECTIVE

The aim of the current study is to establish a relationship between oxidative stress and chemokines with disease activity in MS. We measured the levels of chemokines in the cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients with established oxidative stress.

METHODS

Spinal fluid was obtained from 25 patients with clinically definite MS and 10 control pateints with other neurological disease with consent under an IRB-approved protocol. CSF was examined to ensure absence of red blood cell contamination and stored at -80 degree until use. Chemokines profiles in CSF were evaluated using the Multi-Analyte ELISArray kit from Qiagen. Oxidative stress was measured using biomarkers for lipid peroxidation – isoprostanes (IsoPs) and malondialdehyde (MDA). Oxidized glutathione (GSSG) as well as the total anti-oxidant status (TAS) in CSF was also determined.

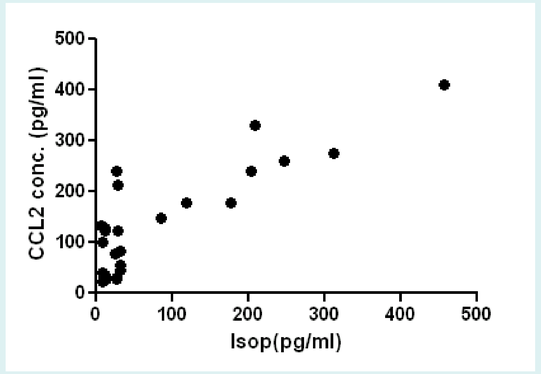
RESULTS

In an initial screen of 25 CSF samples from MS patients, we found significantly increased levels of the chemokines – IL-8/CXCL8, MCP-1/CCL2 and IP-10/CXCL10 in the MS CSF samples with increased oxidative stress.. These chemokines were not increased in the CSF of the control samples with no measurable oxidative stress. Oxidative stress in these samples was established by measuring levels of the lipid peroxidation markers – isoprostane (IsoP) and malondialdehyde (MDA). Oxidized glutathione (GSSG) as well as the total anti-oxidant status (TAS) in CSF was also determined.

Table 1. Chemokine levels in MS Patient samples alongwith oxidative stress marker – IsoP and TAS.

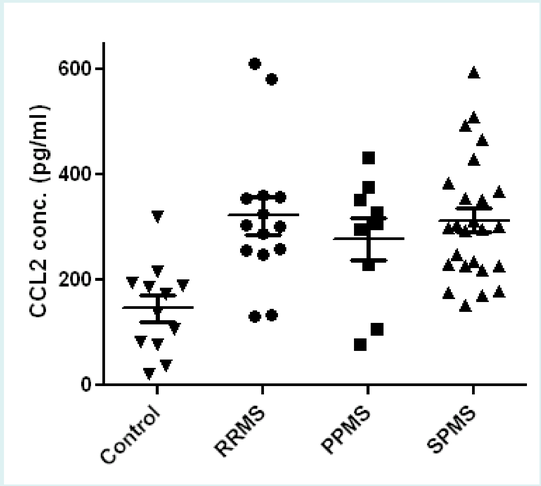
Isop(pg/ml)	TAS (nMol)	IP-10 (pg/ml)	IL-8 (pg/ml)	MCP-1 (pg/ml)
6.7	96.53	21.3	8.841	133.8
8.2	217	11.2	8.841	22.54
8.4	152.11	17.9	9.401	101.01
9.13	217	112	13.878	39.4
29.1	71.63	20.5	7.862	123.7
32	119.29	21.7	19.056	45.267
176.7	112	44.9	20.875	178.45
203.07	125.73	66.778	31.8	239.31
209	97.9	20.425	32.4	330.05
247.4	112.8	32.4	45.082	259.25
312	114.9	309.85	36.407	275.9
457	101.04	37.9	32.768	411.59
31	198	7.5	8.421	83.79
85.1	177	11.2	19.056	146.7
12.7	107.05	4	8.421	27.9
26.7	112.42	13.68	10.8	31.4
28	124.87	3.2	8.841	212.3
11.2	273	33.8	1.565	34.7
12	271	22.54	9.541	122.4
27.4	187	11.01	10.66	27.6
31	105	39.4	12.7	54.9
24.9	122.5	23.7	10.3	76.9
11.3	179	45.267	12.07	127.87
27.4	137.3	4.53	10.2	239.8
117.5	87.6	105.331	27.3	178.5

Figure 1. Chemokine (CCL2/MCP-1) levels in CSF of MS patients correlate with their IsoP levels



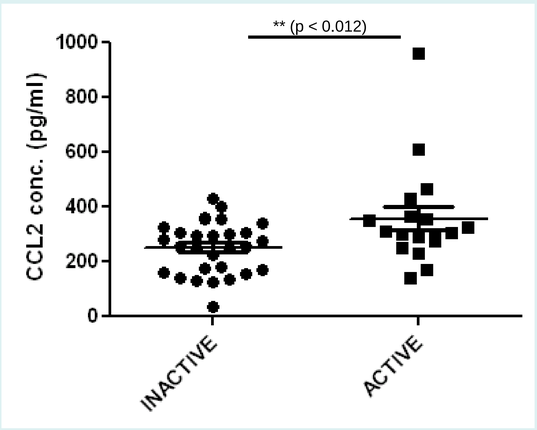
Furthermore, the chemokine levels in these samples correlated with the extent of oxidative stress. For instance, the MCP-1/CCL2 showed a high correlation to the IsoP levels with a Pearson r value = 0.9055.

Figure 2. CCL2/MCP-1 levels in CSF of control and MS patients



It was also found that the CCL2/MCP-1 levels in the MS patient samples varied with disease stage and were significantly higher than those in the healthy control samples. as shown in Figure 2.

Figure 3. CCL2/MCP-1 levels in relation to disease activity in SPMS patients.



Furthermore, a comparison of forty seven clinically active versus stable patients in the SPMS group shows a positive correlation of CCL2 levels with disease activity as shown in Figure 3.

Similar correlations are also being observed with IL-8/CXCL8 and IP-10/CXCL10.

CONCLUSIONS

1. **Levels of the chemokines – IL-8/CXCL8, MCP-1/CCL2 and IP-10/CXCL10 are increased in the CSF of MS patients with increased oxidative stress..**
2. **Chemokine levels in MS sub-groups show significant variation which seems to correlate with disease activity.**
3. **These results indicate the interplay between oxidative stress and immune activity/chemokine production.**

Further studies are warranted to establish the mechanism of this relationship between oxidative stress and chemokine production and how it affects MS disease activity.