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*Oligoclonal bands measured in unconcentrated cerebrospinal fluid
and other biochemical parameters in multiple sclerosis*

The protein content, cells count, presence of oligoclonal bands (OB) in cerebrospinal fluid (CSF) have the clinical importance for the diagnosis of neurological disorders. According to the literature the mean CSF total protein level in MS patients is 450 mg/l (2) and in healthy donors 480 mg/l (ranged from 210 to 2600 mg/l) (11), so there is no significant difference between these values. The normal value of white blood cells (WBC) count in CSF is up to 4 cells/microlitre (1). In MS patients there is a higher level of WBC ranging from 7.6 to 8.2 cells/ μ l (3, 7).

The presence of intrathecal synthesis of IgG antibodies as oligoclonal bands (OB) in the CSF is a very useful indicator of MS. The oligoclonal bands are detected in 88–96% of the patients with clinically defined MS (2, 6, 9, 13). In inflammatory diseases of the CNS, cervical myelopathies and tumors of the CNS oligoclonal bands appear in 21–56% of the patients (2, 13). Intrathecal synthesis of IgG oligoclonal bands was detected in about 68% of the POSMS (possible onset symptoms of MS) group patients (9). The isoelectrofocusing (IEF) in agarose plates, a sensitive method, is used to detect oligoclonal bands in unconcentrated CSF (4). Characteristic bands appear after silver staining (pH range 7–10) (12).

The main interest of the study was the presentation of isoelectrofocusing assay of unconcentrated CSF and silver staining sensitivity used to measure oligoclonal bands. Our interest was also to show the correlation of OB with protein level and WBC count in cerebrospinal fluid obtained from patients with RRMS.

MATERIAL AND METHODS

Patients. Sixty-nine patients with a relapsing-remitting MS were included into the study. In all cases it was at least the second relapse of MS. The first symptoms appeared about 5.5 years earlier (SD 5.2) but there was a difference between the group with and without oligoclonal bands in CSF (7 and 4 years earlier respectively). The median Kurtzke Expanded Disability Status Scale (EDSS) score after admitting to hospital was 3.0 (2.0 to 3.5). The overview of the patient material is presented in Table 1.

Cerebrospinal fluid assays. About 8 ml of CSF were obtained by lumbar puncture. All lumbar punctures were made immediately after admitting patients to hospital and before starting the treatment. 0.2 ml of CSF was incubated with 20 μ l of Giemsa dye over 30 min. in the room temperature to show up the white blood cells. Leukocyte count was examined by using a light micro-

Table 1. Clinical and CSF characterizations of MS patients included into the study

MS	
Number of patients	69
Sex	female 74%
Age	mean 34 years (sd 9.85)
Number of cells in CSF	12 cells/ μ l (range 0 – 64)
Level of protein in CSF	269 mg/l (standard deviation 135.7)
Presence of oligoclonal bands in CSF	72% of patients

scope. After centrifugation of 1 ml of CSF supernatant was frozen at -30°C . The samples were stored from 1 to 14 days.

The isoelectrofocusing and silver staining was carried out according to Mehta (4) and Willoughby (12). The detection of OB in agarose plates (IsoGel agarose IEF plates pH 3–10 BMA products) was made once 8 samples of unconcentrated CSF were obtained from different patients. Briefly, the samples of fourteen- μ l of unconcentrated CSF were run with 1500 V, 15 mA/plate for 45 min. at 10°C (IsoLab Ing. Akron, Ohio). After isoelectrofocusing the agarose plates were consolidated by 30% TCA (trichloroacetic acid). Washing was carried out for two one-hour periods with 5% glycerol. The plates were stained for 3 min. with 0.133% AgNO_3 . The staining was stopped by 10% acetic acid. The CSF total protein level was determined by Lowry assay (5). Statistical comparison between the groups was performed by using an analysis of variance test, Student's t test and correlation's ratio.

RESULTS

A significant number of RRMS patients included into the study were young women. The mean age of patients was 34 years. Sixty-four percent of patients were between 20 to 39 years old (see Fig. 1). Seventy-four percent of all the examined patients were women.

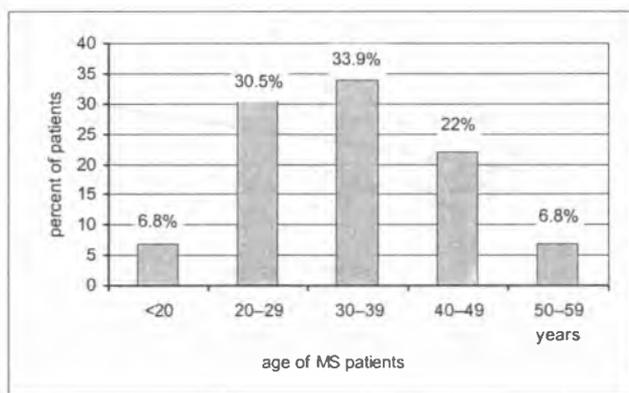


Fig. 1. The age and the sex of MS patients included into the study

The mean value of cells count in CSF was 12 cells/ μ l (standard deviation – SD 13.26). In two patients we observed a very high cells count above 150 cells/ μ l. The mean total protein level in CSF amounted to 268 mg/l (SD 135; range from 61 mg/l to 712 mg/l). The presence of intrathecal synthesis of IgG as oligoclonal bands in CSF was measured in 72% of all patients.

In our study we found a statistical difference ($p=0.05$) between the cells count in patients with the presence of OB and patients, where OB were absent in CSF. In the first group the mean cells count was 15.5 cells/ μ l (SD 16.7) and in the second one the mean was 8.8 cells/ μ l (SD 8.7) (see Fig. 2). There was no significant difference between the total protein level in patients with OB and without OB in CSF (respectively 274 mg/l and 253 mg/l in both groups) ($p=0.05$; analysis of variance test). There was no significance correlation between WBC count and total protein level, $p=0.025$ (Student's t test) (see Fig. 3).

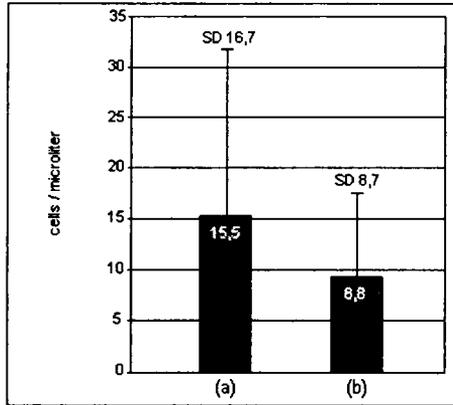


Fig. 2. Cells count in cerebrospinal fluid patients with oligoclonal bands OB (a) and without OB (b)

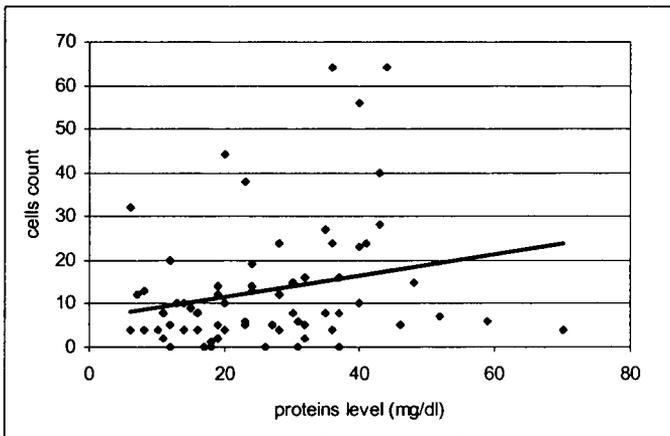


Fig. 3. Correlation between cells count and proteins level in CSF of patients with MS ($r=0.226$)

DISCUSSION

In this study we showed basic parameters of CSF patients with RRMS like cells count, the total protein level and the presence of oligoclonal bands. The majority of all patients in the group were women from 20 to 40 years old. The typical features of MS, such as young age and periods of progression and remission were observed while the study was performed.

The level of WBC was higher than in healthy donors but the difference was of course not significant to make a diagnosis of MS. The increased WBC level is observed in many neurological disorders (e.g. inflammatory diseases of CNS, tumors) and the value is not a characteristic parameter of MS.

We noticed the presence of oligoclonal bands in 72% of the patients. Oligoclonal bands measured in unconcentrated CSF by using agarose plates appeared to be less sensitive than other methods in which polyacrylamide gels and concentrated CSF were used (13). In the cases in which OB were absent there was an opportunity to continue the study by using more sensitive methods like immunoblotting, but those methods were not performed. About 95% patients with CDMS (clinical defined MS) have oligoclonal proteins in concentrated CSF (13).

Comparing the two groups of MS patients: the first one with oligoclonal protein detected in CSF and the second one without oligoclonal protein, the number of WBC in CSF was higher in the first group. By observing an increase of total WBC level in routine CSF examination in MS patients we may expect an increase of B cells level and the level of other cells which facilitate a migration of B cells through blood brain barrier into CSF. In such cases once the isoelectrofocusing of CSF was performed oligoclonal bands were often detected.

CONCLUSIONS

Oligoclonal bands measured in unconcentrated CSF by using an isoelectrofocusing of agarose plates is a very helpful method as indicator of MS, but it appeared to be less sensitive than other methods in which polyacrylamide gels and concentrated CSF were used. The cells count in patients with the presence of OB was higher than in patients without OB in CSF. There was no significant correlation between WBC count and total protein level in CSF RRMS patients.

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SUMMARY

Various biochemical parameters of cerebrospinal fluid (CSF) obtained from sixty-nine patients with RRMS (relapse-remitting MS) were compared in this study. Total protein level, white blood cells (WBC) count and presence of oligoclonal bands (OB) in CSF were examined. The presence of OB in unconcentrated CSF using an isoelectrofocusing assay on agarose gels and silver staining were measured in 72 percent of patients. The average CSF total protein level measured by Lowry assay was 269 mg/l (range from 61 to 712 mg/l). The mean level of white blood cells (WBC) was higher than in general population and it was 12 cells/ μ l. We found an increased level of WBC count among patients with the presence of oligoclonal bands in CSF.

Obecność prążków oligoklonalnych w niezagęszczonym płynie mózgowo-rdzeniowym i inne biochemiczne parametry w stwardnieniu rozsianym

Przedmiotem badań niniejszej pracy było porównanie podstawowych parametrów płynu mózgowo-rdzeniowego pacjentów z nawracająco-remitującą postacią stwardnienia rozsianego (RR-MS). Oceniano poziom białka całkowitego, liczbę leukocytów oraz obecność prążków oligoklonalnych w płynie mózgowo-rdzeniowym. Badając metodą izoelektroogniskowania na żelach agarozowych niezagęszczony płyn mózgowo-rdzeniowy, stwierdzono obecność oligoklonalnego białka u 72% pacjentów. Średni poziom białka całkowitego mierzonego metodą Lowry'ego wynosił 269 mg/l (zakres wartości od 61 do 712 mg/l). Liczba leukocytów była wyższa niż w populacji zdrowych osób i wynosiła 12 komórek/ μ l. Ilość leukocytów była statystycznie wyższa u pacjentów, u których stwierdzono obecność prążków oligoklonalnych.