Charles University in Prague

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Autoreport for Dissertation Work

BIOLOGICAL BEHAVIOUR OF BETA2-MICROGLOBULIN IN LIQUOR IN CLINICALLY DEFINED NOSOLOGICAL UNITS

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Contents

1. INTRODUCTION

1.1. Theme description

The objective of this work, namely of its theoretical part, was to sum up basic information about liquor /its development, formation, composition, its basic and more detailed examination in clinical practice/.

A brief description of liquorological cytological findings including picture documentation.

This work is in more details dedicated to protein beta2-microglobulin.

The introduction brings an abstract of essential description of a molecule of this protein as well as a brief account of its examination. The importance of its examination.

The following part shows methods applied worldwide for this protein examination, the importance of its examination, and conclusions of works published in connection with its examination in different diseases. Conclusions from researches and their importance on establishing the diagnoses, treatment and further prognoses in various nosological units – e.g. in HIV positive patients – have been summarized specifying therapy, prognosis in patients with degenerative CNS disease, inflammatory disease…

The follow-up itself was carried out in a large group of patients – in total 26,378 liquor samples taken between 1999 – 2006, the age limit ranging from 3 to 86 years.

Samples were taken practically all over the whole Czech Republic and elaborated in a biochemical laboratory Na Homolce.

This large group of patients was divided into 4 essential groups according to their diagnoses:

Demylinisation disease, neuroboreliosis, tumours, CNS, others /standard findings and other diagnoses e.g. degenerative disease, vertebrogenous disease/.

An average value of beta2-microglobulin and standard deviation was calculated in individual groups.

Levels of this protein were compared between groups of men and women, at age groups / provided it was possible to compare and statistically compile the values in individual age groups/.

The outcomes were worked into a graphical form.

Conclusion of this work provides a total summarization of the research – the importance of examination of beta2-microglobulin, comparison of protein levels in individual groups according to their diagnoses. The highest values are reached by beta2-microglobulin in the group of tumorous diseases. On significantly high values CNS tumorous disease might be directly considered and further examination procedures might be aimed at this direction.

The level of beta2-microglobulin is very near standard values in demyelinisation diseases.

A different level in men and women can be found in physiological findings.

The extension of the group provides a considerable predicative value – thus no random results are in question.

Findings of this work is definitely not negligible and it makes its way for the following researches and comparisons of behaviour of this significant protein.

1.2. Biological behaviour of beta2-microglobulin in liquor in clinically defined nosological units

Under physiological conditions liquor is a colourless clear liquid found in the ventrical system of the brain, in cisterns and subarachnoidal areas of the brain and medulla.

It is produced in chorioidal plexes. It circulates on from both lateral chambers through foramen Monroi into the IIIrd chamber and through Sylvius ductulus into the IVth chamber. From there it flows through foramina Luschkae into pontocerebellar cornua and through foramen Magendi into cerebromedular cistern. Liquor is resorped in Pacchion granulations and dural sinus into venous circulation.

Functions of liquor:

1. mechanical protection of the brain and medulla against concussions…
2. levelling/balancing intracranial and intravertebral pressure ratios with venous system
3. function of regulation and protection on changes of temperature and atmospheric pressure
4. metabolisms of neurons, removal of products of catabolism
5. immunological processes

An average amount of liquor in adult is about 140 ml, its average daily production is about 500 ml.

Most often lumbal punction is used for taking the liquor. The liquor is taken into 3 test tubes at minimum.

- **Examination**
- – acute - macroscopic description, total protein, cytology, lactate

- primary – macroscopic description, general protein, cytology, lactate and glucoses, albumin, IgA, IgG, IgM, oligoclonal IgG in liquor and serum, specific antidote indexes, number of erytrocytes and presence of haemoglobin
- extended – PCR on HSV encephalitis suspicion, tb, CMV infection, tumour marker, CNS proteins quantification

Metabolism among blood, liquor and nerve tissue is regulated by barriers creating 4 compartments:

1. intravascular
2. intracellular
3. extracellular
4. liquor

There are the following barrier systems among individual compartments:

1. haematoencephalic barrier
2. haematoliquor barrier
3. intra/extracellular barrier

1.3. Biochemical liquor examination

The examination spectrum has still been expanding as a result of development of laboratory methodologies.

In principle, the following should be examined:

- total/general protein level
- glucose level
- immunoglobulin and albumin level assessment

- acute phase proteins assessment

In addition, in extended examination also structural CNS proteins are examined /gamma trace protein, basic/alkaline myelin protein, beta trace protein, tau protein…/

Immunoglobulins are assessed by electrophoretic separating methods – isoelectric focusing.

Based upon the consensus 5 types of potential outcomes on pairing/matching analysis of liquor and serum have been suggested:

1. standard liquor
2. oligoclonal IgG only in liquor
3. oligoclonal IgG in liquor and other identical bands in serum
4. identical oligoclonal bands in liquor and serum
5. monoclonal bands in liquor and serum

1.4. Liquor spectrophotometric examination

This examination provides information on presence of haematogenous pigments in liquor. It is important in haemorrhaegic incidence. It helps differentiate presence of arteficial bleeding.

1.5. Liquor cytology

Cells in liquor are presumably of haemal origin. Under standard circumstances there occur 2 cell types in liquor: lymphocytes, monocytes. Erytrocytes and neutrophil granulocytes are present only as a result of arteficial haemal admixture.

Characteristic cytological pictures in different clinical units have been described.

1.6. Beta2–microglobulin

Beta2–microglobulin is a low-molecular protein made of 99 amino-acids the sequence of which is known. It has an internal disulphidic bond and thus it creates a loop smyčka. It forms a light invariable chain of histocompatible antigens /HLA of Ist class/ against which the post-transplantation immunological intervention is focused.

HLA are synthesised by all vital nuclear body cells in different intensity and located on their cellular surface. They cannot be found on erythrocytes.

There is non-covalent bond between beta2-microglobulin and heavy HLA strand; as a result of metabolism and HLA degradation it is dissociated from heavy chains and then it occurs in a free form in extracellular liquid and in all body fluids, whereas heavy HLA chain is readily degraded.

Beta2-microglobulin level in serum is increasing in different states – in tumours, inflammations, immunological diseases, renal failure,....

Great many studies quantifying beta2-microglobulin in liquor in connection with treatment of HIV positive patients, CNS infectious diseases and degenerative diseases have been published.

2. OBJECTIVE OF THIS WORK

To give reason for examination of beta2-microglobulin level in liquor;

To elucidate the nature of this protein in a theoretical part as well as upon own investigation/monitoring.

To try to find out whether beta 2-microglobulin level depends on a certain nosological unit; whether the level of this protein depends on gender and age.

To support beta2-microglobulin examination in liquor in all taken samples.

3. MATERIAL AND METHODOLOGY

Between 1999 and 2006 a biochemical laboratory Na Homolce examined 23,432 samples of liquor taken from patients from practically all over the CR.

Different general diagnoses were established - meningitis/serous, bacterial/, demyelinisation disease, tumours, polyneuropathy, polyradiculoneuritis...

Samples from patients with vertebrogenous diseases /where there were physiological findings in liquor/ were used as a control group.

Diagnosis confirmation: liquor immunoelectrophorezis, determination of antibodies, CNS morphological examination.

3.1. Methodology:

Beta2–microglobulin level was examined by immuno-turbidimetric method, i.e. monitoring the growth of opacity on bond of an antibody to beta2-microglobulin. Monitoring was carried out on SYNCHRON LX 20 analyser from Beckman company. Applied chemicals were from Bio-Vendor ČR company.

3.2. Statistical processing

It was based on calculation the average values and standard deviations.

4. RESULTS

The file of samples being monitored was made of 26,378 samples of patients at the age ranging from 3 to 86 years the liquor of whom was examined in a biochemical laboratory Na Homolce between 1999 – 2006.

The file was divided into the groups according to their diagnoses:

1. demyelinisation disease – RSM
2. neuroboreliosis
3. tumours
4. others

An average level of beta2-microglobulin and standard deviation was calculated in the examination. Further on, the results of age categories and those of men and women were compared.

Individual groups and results were also compared in graphs/charts.

4.1. Demyelinisation disease - RSM

The group consists of 8,652 examined samples.

The average value of beta2- microglobulin in liquor is 1.885; standard deviation 0.917.

No relation to gender has been proved, age relation seems possible.

4.2. Neuroboreliosis

The group consists of 1,312 examined samples.

The average value of beta2 –microglobulin in liquor is 2.485; standard deviation 5.580

No relation to gender, in children – higher average value of beta2 – microglobulin.

4.3. Tumours

The group consists of 186 examined samples.

The average value of beta2- microglobulin in liquor is 3.202; standard deviation 2.715.

Relation to gender seems proved, lower average value of beta2-microglobulin at lower age categories.

4.4. Standard findings

The average value of beta2- microglobulin in liquor is 1.720; standard deviation 3.490.

5. DISCUSSION

The results confirm difference of beta2- microglobulin level in individual groups.

The highest levels have been found in the group with tumours.

In the group of demyelinisation diseases the values are very near standard values. In this type of disease /provided plasmocytes are not recorded in cytological preparation/ the findings is also near the standard. Final diagnosis is established with the help of electrophoresis. Does the level of beta2-microglobulin also depend on the state of the disease? Could the success rate

of the therapy of RSM disease be assessed according to levels of this protein /as it is in HIV positive patients, and success rate of the therapy assessment/?

Relation of beta2-microglobulin level to gender is obvious in some groups.

6. CONCLUSION

The above given results show the importance and predicative value of beta2-microglobulin examination.

Another research and elaboration of disputable questions in this area is to be considered.

I assume the size of the file being examined provides a high predicative value.

The examinations were carried out at one work place, thus an error on processing individual samples is precluded.

Examination of this protein does not require any special financial means and with respect to its predicative value it should be considered fundamental cerebrospinal fluid examination in the future.

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