Cellular immune response in prognosis of Bell’s palsy and its relation to clinical and electrophysiological findings

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Abstract Objective: To determine the cellular immune response in Bell’s palsy (BP) and its prognostic value in relation to clinical and electrophysiological findings.

Methods: Twenty patients with BP were subjected to: Facial nerve paralysis assessment according to House–Brackmann (H&B) grading system, bilateral facial nerve conduction study with electroneuropsychography (ENoG) quotient calculation, blink reflex, and needle electromyography (EMG) for the affected side; one week from the onset. Before the start of medical treatment, peripheral blood mononuclear cell subsets were analyzed to reveal the percentage of total T cells (CD3 +), T helper/inducer cells (CD4 +), T cytotoxic (CD8 +) and B cells (CD19 +). Patients were followed up by H&B, ENoG and needle EMG up to 3 months from the onset (end point). Fifteen age and sex matched healthy control subjects for the electrophysiological study and laboratory tests were included.

Results: The percentages of CD3 +, CD4 + & CD8 + were significantly depressed in BP patients than in control. CD19 + percentage did not show significant difference between them. On follow up, H&B revealed significant improvement. Neither electrophysiological parameters nor ENoG showed significant difference between initial and follow up assessments. Initial CD4 + percentage correlated negatively with disease duration. While Initial CD8 + percentage correlated positively with follow up compound muscle action potential (CMAP) amplitude of orbicularis oris muscle and ENoG of orbicularis oculi and nasalis muscles. Initial CD19 + percentage correlated negatively with follow up H&B and R1 & R2 responses of follow up blink reflex. Initial CD3 + percentage did not correlate with any of the follow up measures.

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1. Introduction

Bell’s palsy (BP) is primarily an acute 7th cranial nerve paralysis that cannot be attributed to central or peripheral lesions, systemic infections or inflammatory conditions. Several mechanisms have been proposed for the etiology of BP including ischemia, autoimmune reaction and viral infection. Herpes simplex 1 virus (HSV-1) was proved to be a cause of BP by several studies. It is thought to persist as a latent infection. Reactivation of a dormant HSV-1 within the geniculate ganglion (GG) and anterograde transport to facial nerve termini with subsequent inflammation and entrapment of the facial nerve at the meatal foramen was proposed as a pathogenic mechanism in BP. Reactivation of a latent virus can be triggered by surgical trauma, upper respiratory tract infection, fever, dental extraction, emotional distress, exposure to cold, metabolic disorders and immunosuppression. Among the lymphocytes, the role of CD8+ T cells is established where the reduced number of these cells is among the factors that facilitate HSV-1 reactivation. Prevention of reactivation requires a complex interplay among the virus, neurons and immune response. An accumulating evidence supports the view that CD8+ effector T cells employ both lytic granule (perforin)- and IFN γ-dependant effector mechanisms in maintaining HSV-1 latency. The exact role and timing of contribution of lymphocyte subsets during HSV-1 reactivation is still uncertain. On the other hand, a number of case reports have linked recent primary oral HSV-1 infection with unilateral or bilateral facial paralysis. In primary HSV-1 infection a strong immune response evolves composed of virus neutralizing antibodies and antiviral CD4+ and CD8+ T cell response which efficiently inhibits virus replication with induction of virus-specific immunity by the adaptive immune system.

A concern of both patients with BP and their physicians is when and how facial paralysis will be completely resolved. The underlying pathology of the facial nerve is the major prognostic indicator in such patients i.e. demyelination favors a good prognosis whereas axonal degeneration has a bad one. Accordingly, it is assumed that the underlying immune response will determine the severity of inflammation and nerve damage and hence the prognosis of BP. Since lymphocytes are migratory cells i.e. their distribution in the body reflects the rate at which they enter and depart particular sites as well as their local replication, thus these emigrating cells eventually are carried into the bloodstream and traffic among organs according to their interaction with the target tissue. Therefore, in a clinical setting as BP, it seems convenient to study the peripheral blood lymphocytes subsets which reflects their dynamic activity and hence the competence of the immune system. In this context, several studies investigated the lymphocyte subsets in BP with controversial results. Therefore, this study aimed at identifying the pattern of peripheral blood lymphocyte subsets in BP patients compared to healthy controls and their relation to disease prognosis as assessed by functional and electrophysiological outcome measures.

Study design: case-control prospective study.

2. Subjects and methods

Twenty patients; 11 males (55%) and 9 females (45%) with unilateral BP were included in the study. Their ages ranged between 7 and 55 years (mean ± SD: 28.9 ± 15.2). The diagnosis of BP was determined according to criteria described by Taverner: sudden onset, unilateral, complete or partial paralysis of facial muscles; absence of symptoms and signs attributable to nervous system diseases; absence of symptoms and findings attributable to ear and posterior fossa lesions as well as history of trauma to the head or surgery in the face or the parotid region, diabetes mellitus, infiltrative disease and history of irradiation to the neck. Patients with a previous history of Bell’s palsy were excluded from the study.

The study also included 15 age and sex matched healthy control subjects (without a previous history of Bell’s palsy) for the electrophysiological study and laboratory tests. The control group was composed of 8 (53.3%) males and 7 (46.7%) females with an age range of 9–53 years (mean 26.1 ± 10.9).

All patients were subjected to the following:

1. History taking including; demographic data: name, age, sex, occupation, present history: mode of onset, duration of the disease, herpetic eruptions.
2. Full neurological examination.
3. Examination of facial muscles and their grading according to facial nerve grading system of House-Brackman (HB).
4. Electrophysiological examination was performed at 10 to 15 days of the disease onset which included the following:
   a) Bilateral facial nerve motor conduction studies (for patient and control groups).

The facial nerve was stimulated below the ear anterior to the mastoid. The active recording electrode was placed over the frontalis, orbicularis occuli (OOc), nasalis and orbicularis oris (OOr) muscles. The reference electrode was placed over the standard corresponding point. The ground electrode was placed at the wrist. The compound muscle action potential (CMAP) latency and amplitude were recorded for each studied muscle. In the patients’ group, the ratio of the amplitude of CMAP on the affected side to that on the healthy side was calculated as a percent (electroneurographic quotient i.e. ENoG quotient).

Conclusion: Decreased percentage of peripheral blood CD3+, CD4+ & CD8+ in BP patients emphasizes the role of cell mediated autoimmune pathogenesis in the acute stage of the disease. These cells have a prognostic significance for prediction of the disease duration and outcome. Analysis of T lymphocytes subsets may provide an additional parameter to differentiate patients with favorable from those with poor prognosis.
Electroneurography served as an index of degree of nerve involvement.\textsuperscript{29}

\[ \text{ENoG quotient} = \frac{\text{Amplitude of affected side}}{\text{Amplitude of healthy side}} \times 100 \]

Regarding the control group, calculation of ENoG quotient was carried out according to the following formula:

\[ \text{ENoG quotient} = \frac{\text{side with smaller CMAP amplitude}}{\text{side with larger CMAP amplitude}} \times 100 \]

b) Blink reflex\textsuperscript{28}

Recording was performed from both sides simultaneously using 2 channels. Recording settings were: sweep speed 10 ms/division, gain 200 μV/division and analysis time 100 ms.

5. Needle electromyography (EMG) of OOc and OOr muscles of the affected side was carried out after 10–15 days of the onset.\textsuperscript{30} Patients with axonopathy should have both EMG findings of axonal degeneration in the acute stage (denervation potentials and decreased recruitment) and ENoG less than the mean-2SD of that of the control group, otherwise demyelination is considered.\textsuperscript{28}

6. EDTA blood samples were obtained from all patients and control group before the start of medical treatment. Peripheral blood lymphocyte subsets were analyzed with flow cytometry (Becton–Dickinson, Erembodegem, Belgium) and monoclonal antibodies labeled with either fluorescein isothiocyanate or phycoerythrin (CD3/FITC, CD19/PRE, CD4/FITC and CD8/PRE) to reveal the percentage of total T cells (CD3+), B cells (CD19+), T helper/inducer cells (CD4+) and T cytotoxic cells (CD8+).

All patients were treated within the first 72 h of symptoms onset, after obtaining blood sample, with oral acyclovir (400 mg five times daily) and oral prednisolone (at a dose of 40–60 mg daily)\textsuperscript{41} that was gradually withdrawn.

Patients were followed up by HB grading system, facial nerve motor conduction studies, ENoG, needle EMG and assessment of T-cells percentage on recovery or up to 3 months of the onset (as an end point).\textsuperscript{32} The study was approved by the Local Ethics Committee, Faculty of Medicine, Alexandria University. All patients and control groups were informed about the nature of the study and written acceptance consent was signed by each participant.

Statistics: Description of the sample was expressed as frequencies and percentages. Distribution of all variables was evaluated using Skewness test. All variables were normally distributed except for the initial and follow up latency and amplitude of the affected OOc and OOr muscles respectively as well as the initial and follow up peripheral blood CD3+ and CD19+. For normally distributed variables, Student test was used to compare between patients and controls, while paired t test was used for initial and follow up comparisons. For other variables, Mann–Whitney test was used for comparison between patients and controls and Wilcoxon test for initial and follow up comparison. Correlation between variables was examined by Spearman’s test. SPSS v 11.0 was used to perform the statistical analysis. Cut off values were calculated as the mean ± 2SD of controls for electrophysiological parameters. \( P \) is significant if \( \leq 0.05 \).

3. Results

No statistical significant difference in age was found between patients and controls (\( t = 0.578, P = 0.568 \)). Eleven patients (55%) had right side BP and 9 patients (45%) had left side lesion. None of the patients complained of hyperacusis or disturbed taste sensation i.e. all had distal facial nerve affection. The triggers for the disease as stated by the studied patients were exposure to cold, emotional stress and a preceding upper respiratory tract infection. Mean disease follow up duration was 5.36 ± 4.78 weeks. According to HB grading system; 5 patients (25%) were grade II, 3 patients (15%) were grade III, 5 patients (25%) were grade V, and 7 patients (35%) were grade VI at initial evaluation. On follow up evaluation, 7 patients (35%) were grade I, 5 patients (25%) were grade II, 2 patients (10%) were grade III, 3 patients (15%) were grade V, and 3 patients (15%) were grade VI. Table 1 shows the comparison between patients and controls regarding the ENoG findings as well as the ENoG cut off values (mean of the control value-2SD) of the studied facial muscles. There was a statistically significant decrease of the ENoG of frontalis, nasalis and OOr of patients compared to controls. \( (p \leq 0.05) \) (Fig. 1).

Table 2 shows the mean values of initial and follow up HB grading system. A statistical significant improvement was found in HB grading system at follow up evaluation (\( P = 0.001 \)). It shows also the mean amplitude and latency of the facial nerve CMAP recorded from the frontalis, OOc, nasalis, OOr muscles of the affected side among the studied patients at initial and follow up evaluation. Neither the mean latency nor the mean amplitude of the recorded CMAP showed a statistically significant difference on comparing the initial to the follow up assessment. Similarly, ENoG did not show significant difference between initial and follow up values.

Individual data analysis revealed that 5 patients (25%) had demyelinating pathology (they recovered completely before 3 months) and 15 patients (75%) had axonal lesion based on cut off values of ENoG and EMG findings as well as the follow up HB grading system. Table 3 shows the mean values of peripheral CD3+, CD4+, CD8+ and CD19+ mononuclear cell subsets among patients and control groups at initial evaluation. The initial percentage of CD3+, CD4+ & CD8+ subsets was significantly depressed in BP patients group compared to age matched control subjects (\( p \leq 0.05 \)). However, the percentage of CD19+ subset did not show a significant difference between patients and control groups (\( p = 0.69 \)).

Moreover, comparison between BP patients with demyelinating pathology and those with axonal lesion regarding the mean initial percentage of peripheral blood T cells revealed no statistical significant difference (CD 4+: \( t = -0.6, P = 0.558 \), CD8+: \( t = 1.596, p = 0.138 \) and CD3+: \( Z = -0.681, p = 0.496 \)), while there was a statistically significant increase in the mean initial percentage of CD19+ in patients with demyelination compared to those with axonopathy (\( Z = -2.632, p = 0.008 \)). Spearman’s correlation revealed a negative correlation between the initial percentage of CD4+ subset with disease follow up duration (\( r = -0.882, P = 0.02 \)). Initial percentage
of CD8+ subset correlated positively with follow up CMAP amplitude of OOOr muscle (r = 0.841, P = 0.04) on the affected side and positively with follow up ENoG of OOC and nasalis muscles (r = 0.894, P = 0.04 for each). Initial percentage of CD19+ subset correlated negatively with follow up HB grading system (r = −0.807, P = 0.003) and negatively with latencies of ipsilateral R1, R2 and contralateral R2 responses of follow up blink reflex on the affected side (r = −1.0, P = 0.001 for each). Initial CD3+ percentage did not correlate with any of the follow up measures.

4. Discussion

Among the proposed mechanisms underlying BP which may be directly relevant to the immune system are the reactivation of HSV-1,9–12 and the less commonly considered; the autoimmune mechanism.3,21 In the present study, the studied BP patients were assumed to have reactivation of a latent herpes virus as a probable pathogenic mechanism. None of the studied patients was suffering from primary herpes infection (or any other illness) at the time of presentation of BP which ruled out the possibility of the primary or recent HSV-1 infection with concomitant BP. In the context of autoimmunity, Abramsky et al. suggested that in BP there is in vivo sensitization of lymphocytes to self protein called neuritogenic basic protein and concluded that an immunological process similar to Guillain-Barr syndrome (GBS) occurs in BP and even the latter could represent a mononeuritic variant of GBS.3 In the present study neither specific viral nor specific neural markers were assessed, therefore, both mechanisms will be adopted to explain the results of the study.

Our study revealed a decrease of the mean percent of the components of cellular immunity (CD8+, CD4+ and Total CD3+) among the studied patients while CD19+ percent (B cells) was slightly increased in patients though not statistically significant compared to control. The interactions between viruses and the host immune system are not only complex but also critical in determining the outcome of infection and prevention strategies.3 In the target tissue (GG in BP) where the virus resides in a latent state, infiltrating virus-specific
In the present study, comparing the initial T cell percentage among patients with demyelination and those with axonal degeneration revealed that both groups had comparable values of T cells but patients with demyelination had a significant increase in the initial percentage of B cells which may account for the relatively favorable prognosis of such patients. Although the role of B cells was not well addressed in HSV-1 latency and reactivation, yet some studies demonstrated the beneficial role of B cells in controlling the persistent viral infection. Stress-induced HSV-1 reactivation results from a transient compromise of CD8+ T cells surveillance. Thus, the humoral responses act in concert with cellular immunity to control primary and latent viral infections. Yet, it can be assumed that patients with demyelinating facial nerve pathology (with increased percent of CD19+) had a less inflammation, less edema around the nerve, less compression and hence the possibility of axonal degeneration is less. This deduction was supported by the negative correlation between CD19+ in patients group and the follow up HB grading score emphasizing the role of CD19+ B cells in controlling the persistent viral infection.

Studying the changes of the immune system in patients with BP was a point of interest for many researches with controversial results regarding the findings and interpretations. Jonsson et al. analyzed T lymphocytes and their subsets in 20 BP patients and found a decrease in the total T cell percentage and T helper cells (CD4+) (as the present study). But unlike ours they found no change in the percent of cytotoxic T cells (CD8+). After 4–6 weeks these depressions were restored to normal levels. In our study all increased on follow up but...
reaching a significant level only for the total percent of CD3+ (data not shown). In another study performed by Aveil et al. the results were similar to ours.21 Also Vedeler et al. found a significant decrease of the mean percent of the active and total T cells in the first week in BP patients among whom 8 patients showed a decreased percent of T suppressor cells and all returned to the normal values after 3 months of the disease onset.49 On the other hand, Kaygusuz et al. studied the role of viruses and cellular immune response in idiopathic facial palsy. They demonstrated that the percent of CD4+ (similar to our results) and CD4+/CD8+ ratios was lower while the percent of natural killer (NK) cells and CD8+ percent was higher than controls (unlike our results) and CD19+ percent was normal (similar to the present study). They suggested that this pattern was similar to viral reactivation. Alternatively, Tekgul et al. found decreased CD19+ cells (unlike ours) and decreased CD8+ T cells in children with BP. They suggested that the underlying mechanism was an autoimmune process resulting from decreased viral clearance due to decreased CD19+ as well as delayed viral clearance due to viral induced CD4+ lymphopenia with subsequent autoimmune process.32 Thus it can be concluded from these studies (including our study) that there is no stereotyped consistent cellular immune response among BP patients and no consensus about the underlying operating mechanism(s). These differences may be attributed to the variability in the designs of these studies. However, this conflict may have its implication on management of those patients.

Although the studied patients exhibited significant improvement of their mean follow up HB score, yet the ENoG, blink reflex and CMAP of the facial muscles improved but not to a significant level. In several studies, histological analysis of facial nerve in BP revealed a mixed lesion of various nerve injuries i.e. intact, demyelinated and degenerated nerves.50–52 It was also found that inflammation of the nerve initially leads to reversible neurapraxia but ultimately Wallarian degeneration occurs.48 Thus, it could be assumed that the recovery of the demyelinated fibers enhanced by medical treatment with or without a possible regeneration of the degenerated fibers (if the conditions were favorable and the pathology was axonotomesis), all of which can improve the function sizably particularly if demyelination is the predominant pathology. On the contrary, improvement of CMAP and ENoG depends solely on regeneration of the degenerated fibers which may take a longer time and need optimal tissue conditions to take place. Hence, rapid and sizable improvement of CMAP and ENoG is unlikely compared to HB. This assumption is confirmed and can be explained by the significant improvement of HB score in patients with axonopathy on follow up assessment (data not shown, \( P = 0.014 \)). Moreover, the inherent nature of ENoG makes it susceptible to variabilities including impedance, electrode location, skin resistance, different distribution of muscle mass and the patient’s tolerance level to the stimulus intensity.27,53 Therefore its results should be interpreted cautiously. The discrepancy between the improvement of HB and ENoG was further confirmed by the lack of correlations between the follow up HB and ENoG among patients.

Blink reflex has the advantage over ENoG and facial nerve conduction in being able to examine the proximal part of the facial nerve in BP.28 However, there was no significant improvement on comparing its initial and follow up parameters. This can be explained on the same basis as ENoG i.e. the concept of a mixed nerve lesion in BP. It is to be noted that some patients who were grade VI on HB had initial normal latencies of the blink reflex. This may be explained by the presence of few intact nerve fibers which could convey impulses but were not sufficient to perform functional movement. Our results regarding ENoG were consistent with that of Honda et al. who found that the ENoG recovery tended to be delayed compared to clinical recovery in BP.51 Also in another study ENoG failed to provide accurate information regarding the prognosis or the rate of recovery in BP as it had no correlation with the probability of recovery in such patients. However, unlike our results R1 of the blink reflex paralleled the recovery of facial paralysis well.51–55 In this study, patients were divided into those with demyelination, and those with axonal degeneration of the facial nerve according to the initial electrophysiological study which was confirmed later on by improved HB grading on follow up. Four patients with demyelination recovered within 7 to 10 days, while the remaining one recovered completely after 8 weeks. It is to be noted in this study that there was uneven involvement of the facial nerve branches as far as the pathology is concerned (judged by the EMG and ENoG) i.e. some patients showed features of axonopathy in some muscles and demyelination in others and even one patient with demyelinating lesion and who recovered completely after 10 days, had a significant decrease in ENoG of the lower face muscles namely, nasalis and OOr. This implies that ENoG may give false positive results denoting axonal degeneration (particularly in absence of EMG abnormalities).

Some follow up electrophysiological parameters were correlated with the initial percentage of the immune cells in peripheral blood of the studied patients signifying some prognostic value of these cells. The initial CD8+ T cells was positively and significantly correlated with the follow up values of OOr CMAP amplitude and the ENoG of nasalis and OOr muscles of the affected side. This emphasizes the role of CD8+ cells in clearing the virus after reactivation (a window of opportunity for the immune system) and improving inflammation and hence promoting recovery of the nerve i.e. increasing the number of functioning fibers and hence increasing the ENoG and CMAP. Also the initial CD4+ T cells were significantly and negatively correlated with the disease duration implying that increased percentage of CD4+ cells will promote recovery by virtue of its role in controlling viral reactivation or autoimmunity as proposed mechanisms for BP and thus shorten the disease duration. From this study it can be concluded that decreased percentage of the peripheral blood CD3+, CD4+ & CD8+ subsets in BP patients together with increased B subset in patients with demyelination emphasize the role of cell mediated and humoral immunity in the pathogenesis of BP. These cells have some prognostic significance regarding disease duration and facial nerve recovery. Analysis of T lymphocytes subsets (especially B cells) may provide an additional parameter to differentiate patients with favorable from those with poor prognosis.

References


