CLINICAL STUDY

The natural history of macroprolactinaemia

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Abstract

Objective: Macroprolactinaemia is a condition in which serum prolactin (PRL) consists mainly of large molecular weight PRL (macroPRL). The aim of this study was to examine the natural history of macroprolactinaemia.

Design and participants: Six hundred and fifty-four hospital workers participated in this study, including 27 subjects with macroprolactinaemia and 627 controls. MacroPRL and serum PRL concentrations were evaluated over a 4-year period. The ratio of macroPRL was examined by the polyethylene glycol (PEG) method and gel filtration chromatography. IgG-bound PRL and anti-PRL autoantibodies were examined by protein G and ¹²⁵I-PRL binding studies respectively.

Results: Over the 4 years of the study, all 27 macroprolactinaemic subjects had persistent macroprolactinaemia without the development of raised free PRL, while none of the 627 controls developed macroprolactinaemia. The ratios of PEG-precipitable PRL and IgG-bound PRL did not significantly change, but ¹²⁵I-PRL binding ratios significantly increased. As a whole, total and free serum PRL concentrations did not significantly change in subjects with macroprolactinaemia over the 4-year period. However, hyperprolactinaemia developed in five of the 18 macroprolactinaemic subjects who were initially normoprolactinaemic along with an increase in anti-PRL autoantibody titres. One of the remaining nine macroprolactinaemic subjects who were initially hyperprolactinaemic showed a decrease in serum PRL concentrations, which occurred concomitantly with a decrease in the anti-PRL autoantibody titre.

Conclusions: Macroprolactinaemia may develop before middle age and is likely a chronic condition leading to hyperprolactinaemia.

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Introduction

Serum prolactin (PRL) consists of several molecular forms that can be separated by gel filtration chromatography. The main component is usually little PRL, monomeric PRL with a molecular mass of 23 kDa, but there are other minor forms including big PRL (dimeric PRL) and big–big PRL (macroPRL) (1, 2). Macroprolactinaemia refers to a condition where macroPRL, with a molecular mass > 150 kDa, is predominantly present in the sera (3, 4, 5, 6, 7, 8, 9, 10). The prevalence of macroprolactinaemia is estimated to be 10–26% in patients with hyperprolactinaemia (4, 5, 6, 7, 8, 9) and 3.68% in the general population (10). MacroPRL is mainly a complex of PRL with IgG, especially anti-PRL autoantibodies (3, 10, 11, 12, 13, 14, 15). Hyperprolactinaemia is more frequently observed in subjects with macroprolactinaemia probably because there is a delayed clearance of macroPRL due to the large molecular size (10, 16).

The natural history of macroprolactinaemia is currently unknown. It is unclear when macroprolactinaemia

develops in life and whether the PRL status returns to normal later in life. It is also unclear whether individuals with macroprolactinaemia and normal initial serum PRL levels (normoprolactinaemia) eventually develop hyperprolactinaemia.

This study was designed to elucidate the natural history of macroprolactinaemia. We examined over a 4-year period whether any new cases emerged in non-macroprolactinaemic subjects and whether hyperprolactinaemia developed in macroprolactinaemic subjects who were initially normoprolactinaemic.

Subjects and methods

Subjects

Macroprolactinaemia was screened in 654 hospital workers (520 women and 134 men aged 37.4 ± 9.7 years) in 2007, and 27 subjects were diagnosed with macroprolactinaemia. MacroPRL and serum PRL concentrations were evaluated in the 27

macroprolactinaemic subjects (21 women and six men) and the remaining 627 non-macroprolactinaemic subjects (499 women and 128 men) in the years 2007, 2009 and 2011. Blood samples were collected in the morning, and the serum was separated and stored at -80°C until use. This study was approved by the Clinical Research Review Board of Kobe City General Hospital.

Polyethylene glycol precipitation and PRL assays

To determine free PRL concentrations, serum samples (50 μl) were mixed vigorously with 50 μl cold polyethylene glycol (PEG; molecular weight 6000, 25% in water) and centrifuged at 9100 *g* for 10 min to remove macroPRL. The supernatant was diluted tenfold with phosphate buffer (0.01 mol/l, pH 7.0) containing 0.4 mol/l NaCl and 0.1% BSA (final serum dilution 20-fold). Serum samples were treated identically, but with water instead of PEG, to determine total PRL concentrations. Free PRL was defined as the PRL concentration in the supernatant after PEG precipitation, and total PRL was defined as the PRL concentration in the water-treated sample. The PEG-precipitable PRL (%), which represents the amount of macroPRL, was calculated as follows: $(\text{total PRL} - \text{free PRL}) / \text{total PRL} \times 100$. PEG precipitation of $>60\%$ (recovery $<40\%$) was used as the cut-off value for the diagnosis of macroprolactinaemia.

The concentrations of PRL were measured in duplicate using an enzyme immunoassay (EIA) for human PRL (hPRL) as previously described (15). In brief, serum samples were incubated with polystyrene balls (Precision Plastic Ball Co., Chicago, IL, USA) that were coated with anti-hPRL antiserum (NIDDK-anti-hPRL IC-5) at 37°C for 6 h. After washing, the balls were incubated with affinity-purified anti-hPRL Fab'-HRP conjugate at 4°C overnight followed by incubation at 20°C for 6 h. After washing, the peroxidase activities bound to the balls were assayed by an enzyme reaction using 3-(*p*-hydroxyphenyl)propionic acid (Aldrich Chemical Co. Milwaukee, WI, USA) as a substrate, followed by detection of the fluorescence intensity using a spectrofluorophotometer (FP-6200ST, Jasco Co., Tokyo, Japan) with excitation at 320 nm and emission at 405 nm.

hPRL-RP-2 (NHPP, Torrance, CA, USA) was used as a RP. Serum PRL concentrations were logarithmically transformed to get the distribution close to normal (17). The reference intervals for total and free PRL were determined using all samples from the participants of this study except for those with extreme values as judged by Smirnov test for extreme value (18). According to the test, the observed value (X_n) is standardised by the formula: $T_n = (X_n - \text{mean}) / \text{s.d.}$, and compared with the tabulated value corresponding to the sample size. If the observed value exceeds the

tabulated value, it is considered to be an outlier. The tabulated value ($P < 0.05$) for 654 samples is 3.76; therefore, the extreme value X_n was determined by the formula: $(X_n - \text{mean}) / \text{s.d.} > 3.76$. As the ln-transformed total and free PRL values in all 654 samples were 5.24 ± 0.60 and 4.79 ± 0.55 respectively, ln-transformed total PRL concentrations >7.50 and ln-transformed free PRL concentrations >6.86 as calculated according to the above formula were judged as outliers. Thirteen data of total PRL concentrations and 12 of free PRL concentrations were excluded from the total of 654 data to determine the reference intervals. Ln-transformed total and free PRL concentrations in the remaining samples (641 for total PRL and 642 for free PRL) were 5.18 ± 0.55 and 4.73 ± 0.50 respectively. The 95% reference interval for ln-transformed total PRL was calculated as $5.18 - (1.96 \times 0.55) - 5.18 + (1.96 \times 0.55)$ and back transformed these values as 60–522 mIU/l. Similarly, the 95% reference interval for free PRL was determined as 43–302 mIU/l. The PRL concentration can be converted from mIU/l to $\mu\text{g/l}$ using a conversion factor of 1/35. The limit of detection was 0.175 mIU/l. The intra- and interassay coefficients of variation were 4 and 5% respectively, using a serum sample with a total PRL concentration of 178.5 mIU/l.

Measurement of IgG-bound PRL

To determine the ratio of IgG-bound PRL, protein G Sepharose (GE Healthcare, Uppsala, Sweden), which characteristically binds IgG, was used as described previously (15). Serum samples (100 μl) were applied to the protein G columns equilibrated with 0.02 mol/l sodium phosphate buffer (pH 7.0), and the unbound proteins were washed with the same buffer (4 ml). The bound PRL-IgG complex was eluted with 4 ml of 0.1 mol/l glycine-HCl buffer (pH 2.7), and the eluate was immediately neutralised with 1 mol/l Tris-HCl buffer (pH 9.0). The PRL concentrations in the unbound and bound fractions were measured using the EIA. The ratio of IgG-bound PRL (%) was calculated using the equation: $\text{PRL in the bound fraction} / (\text{PRL in the unbound fraction} + \text{PRL in the bound fraction}) \times 100$. A small amount of PRL was bound to protein G in control sera, which may be due to non-specific binding (mean $\pm$ s.d.: $1.3 \pm 0.4\%$). The mean $+ 1.96 \times$ s.d. in 20 non-macroprolactinaemic sera was used as the cut-off value for the presence of IgG-bound PRL; that is, a ratio $>2.1\%$ was judged to be IgG-bound PRL positive.

^{125}I -PRL binding study

Anti-PRL autoantibodies were measured in a ^{125}I -hPRL binding study. hPRL (NIDDK-hPRL-I-8) was radioiodinated with Na^{125}I (PerkinElmer, Waltham, MA, USA) using an IODO-GEN pre-coated iodination tube (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Serum (40 μl) was incubated with

10 μ l 125 I-PRL (2.0×10^4 c.p.m.) at 37 °C for 1 h. Then, 50 μ l of 25% cold PEG solution was added and mixed vigorously. The mixture was centrifuged and the pellet was washed once with 500 μ l of 12.5% PEG. The mixture was centrifuged and the radioactivity of the pellet was measured using a gamma counter (wizard 3, PerkinElmer). A small amount of 125 I-hPRL was precipitated with PEG, which may be due to non-specific binding (mean $\pm$ s.d.: $4.4 \pm 1.7\%$). The mean $\pm 1.96 \times$ s.d. in 20 non-macroprolactinaemic sera was used as the cut-off value for the presence of anti-PRL autoantibodies; that is, a ratio $> 7.8\%$ was judged to be anti-PRL autoantibody positive. The 125 I-PRL binding study was performed in all samples simultaneously to avoid different background values due to iodination on different occasions.

Gel filtration chromatography

Gel filtration chromatography was performed using a 1×60 cm column of Ultrogel AcA 44 (IBF, La Garenne, France), equilibrated with 0.01 mol/l sodium phosphate buffer (pH 7.0) containing 0.1 mol/l NaCl, 0.1% BSA and 0.01% NaN_3 . Samples (100 μ l) were applied on the column, and 1 ml fractions were collected for PRL determination. The column was calibrated with various molecular weight markers (Sigma).

Statistical analysis

Values are expressed as mean $\pm$ s.d. and a $P < 0.05$ was considered significant. Paired t -test was used to compare the parameters of macroPRL and serum PRL concentrations in the years 2007, 2009 and 2011.

Results

The levels of macroPRL and the other parameters over a 4-year period

A total of 654 hospital workers were followed for 4 years to examine the natural history of macroprolactinaemia. At the beginning of the study, 27 of the 654 subjects were diagnosed with macroprolactinaemia and the remaining 627 subjects were not and served as controls. Over a 4-year period, all the 27 subjects remained macroprolactinaemic, and none of the 627 controls developed macroprolactinaemia. The PEG-precipitable PRL ratio, which roughly represents the ratio of macroPRL in the serum PRL, did not change significantly in subjects with macroprolactinaemia (Fig. 1a). All subjects with macroprolactinaemia had IgG-bound PRL with various ratios that did not change significantly over a 4-year period (Fig. 1b). The 125 I-PRL binding ratios, which represent the titres of anti-PRL autoantibodies, significantly ($P < 0.001$) increased in 2009 and 2011 compared with the ratios in 2007

Figure 1 The levels of the parameters of macroprolactin and serum PRL concentrations in subjects with macroprolactinaemia over a 4-year period. The ratios of PEG-precipitable PRL (a), IgG-bound PRL (b) 125 I-PRL binding (c) and total and free serum PRL concentrations (d) in 27 patients with macroprolactinaemia over a 4-year period are shown. Each value is shown as a filled circle. PEG-precipitable PRL ratio $> 60\%$ was used as the cut-off value for macroprolactinaemia (a). Shaded areas in (b) and (c) indicate the mean $\pm 1.96 \times$ s.d. in 20 controls. Shaded areas in (d) indicate the mean $\pm 1.96 \times$ s.d. in all participants of this study excluding 13 for total PRL and 12 for free PRL because of their extreme values. * 125 I-PRL binding ratios significantly ($P < 0.001$) increased in 2009 and 2011 compared with those in 2007.

(Fig. 1c). The number of subjects with macroprolactinaemia, whose sera bound more than 7.8%, increased from 13 in 2007 to 21 in 2009 and 2011.

Total and free serum PRL concentrations over a 4-year period

Initially, nine of the 27 macroprolactinaemic subjects had high total serum PRL concentrations (> 522 mIU/l), and seven of the nine hyperprolactinaemic subjects had normal free serum PRL concentrations (< 302 mIU/l). Two subjects had slightly elevated free PRL concentrations (385 and 420 mIU/l). As a whole, total and free serum PRL concentrations did not significantly change in subjects with macroprolactinaemia over a 4-year period (Fig. 1d).

Hyperprolactinaemia developed in five of the 18 macroprolactinaemic subjects who were initially normoprolactinaemic (cases 1, 2, 3, 4 and 5 in Fig. 2) along with an increase in anti-PRL autoantibody titres. One of the nine macroprolactinaemic subjects with initial hyperprolactinaemia (case 6 in Fig. 2) showed a decrease in serum PRL concentrations, which occurred concomitantly with a decrease in the anti-PRL autoantibody titre.

Figure 3 shows the molecular forms of serum PRL on gel filtration chromatography in cases 1 and 6, whose serum total PRL concentrations increased and decreased more than twofold respectively, over

a 4-year period. In case 1, the 45 kDa PRL started to increase first followed by an increase in both macroPRL and 45 kDa PRL and a decrease in 23 kDa PRL. In case 6, 45 kDa PRL was not observed and both macroPRL and 23 kDa PRL decreased in parallel.

Discussion

In this study, not a single new case of macroprolactinaemia emerged and all subjects with macroprolactinaemia maintained their status over a 4-year period. Hyperprolactinaemia developed along with an increase in anti-PRL autoantibody titres on an individual basis.

Macroprolactinaemia is a very common condition in patients with hyperprolactinaemia (4, 5, 6, 7, 8, 9) and also in the general population (10). However, it is unknown when it begins and ends. At the beginning of this study, 27 of the 654 subjects were already macroprolactinaemic while the remaining 627 subjects were not. No new cases of macroprolactinaemia emerged in a fairly large population of non-macroprolactinaemic subjects during the 4 years. Although subjects in this study may be too old to determine when macroprolactinaemia began, the current data suggest that macroprolactinaemia is likely to begin before middle age. This assumption is supported by a few reports that

Figure 2 Serum PRL concentrations and anti-PRL autoantibody titres over a 4-year period. Macroprolactin (grey bars) and free PRL (white bars) concentrations and anti-PRL autoantibody titres (filled circle) are shown in six subjects with macroprolactinaemia who developed hyperprolactinaemia (cases 1, 2, 3, 4 and 5) and normoprolactinaemia (case 6) over a 4-year period.

Figure 3 Gel chromatographic profiles of serum PRL in two cases with macroprolactinaemia over a 4-year period. Gel filtration chromatographic patterns of serum PRL in cases 1 and 6 in 2007 (filled circle), 2009 (filled square) and 2011 (open circle) are shown. Case 1 developed hyperprolactinaemia; in contrast, case 6 developed normoprolactinaemia.

demonstrated the presence of macroprolactinaemia in childhood and adolescence (19, 20, 21).

There are two reports on the natural history of macroprolactinaemia. Wallace *et al.* (22) followed 51 patients for 10 years and claimed macroprolactinaemia is a benign variant. They showed that serum PRL levels in hyperprolactinaemic patients with macroprolactinaemia were relatively stable in the long term, but free PRL levels in these patients were not shown. We previously reported that the ratios of PEG-precipitable PRL, IgG-bound PRL and ^{125}I -PRL binding were relatively stable during long-term follow-up in 12 hyperprolactinaemic patients with macroprolactinaemia (15). We could not, however, analyse the change in serum PRL concentrations caused by macroPRL in our previous study because about 40% of these patients had hyperprolactinaemia due to causes other than macroPRL. In this study, most macroprolactinaemic subjects had normal serum-free PRL concentrations, which strongly indicates only macroPRL is responsible if hyperprolactinaemia develops. Our finding that hyperprolactinaemia developed in five macroprolactinaemic subjects, who were initially normoprolactinaemic along with an increase in anti-PRL autoantibody titres, suggests that autoantibodies are, at least in part, responsible for the development of hyperprolactinaemia. This assumption is reinforced by the finding that serum PRL concentrations returned to normal in one macroprolactinaemic subject, who was initially hyperprolactinaemic, concomitantly with a decrease in anti-PRL autoantibody titres. It is possible that autoantibody-bound PRL accumulates in the circulation over a long term because of delayed clearance due to its large molecular size (16).

In conclusion, macroprolactinaemia may develop before middle age and is likely a chronic condition leading to hyperprolactinaemia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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