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研究課題名（和文）　小児・配偶者・高齢者に対する虐待の法医病理学的証明に関する研究

研究課題名（英文）　Study on Forensic Pathological Demonstration of Child, Partner and Elder Abuse

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研究成果の概要：各種虐待の法医病理学的証明法の開発を目的として，まず高齢者虐待例の胸腺・副腎重量を対照例と比較したところ，重量に有意差を認めなかった。しかしながら，虐待期間が３ヶ月未満の虐待例の副腎重量は対照例に比して有意に増加していた。次に，各種臓器に浸潤する白血球数を検討したところ，特に肺・肝臓において虐待例で有意に増加しており，高齢者に対する身体的虐待の法医病理学的証明法として有用である可能性が示唆された。

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１．研究開始当初の背景

近年，小児・配偶者・高齢者に対する虐待が社会問題となり，各虐待防止法も施行されているが，今までのところ社会的対策は充分とはいえない。法医解剖においても虐待被害者の死亡例を少なからず経験し，特に近年では高齢者の例が増加している印象がある。そこで今回，虐待の法医病理学的証明，虐待の程度と期間の推定，身体機能に及ぼす影響などの事項を究明する研究を企画した。

われわれは 1991 年，小児の身体的虐待に基づく広範な皮下出血の出血血液から溶出した hemoglobin が循環血に入り，腎臓の近位尿細管細胞内に沈着することを明らかにし，小児虐待の補助診断に有用であることを示唆した。小児虐待が身体に及ぼす変化に関しては，われわれの報告の他にも，1990 年初頭に胸腺の退縮，ストレス蛋白質 ubiquitin の出現などが報告されてきた。しかしながら，最近では小児虐待が身体に及ぼす影響に関する報告は比較的少ない。また，配偶者や高齢者に対する虐待が被害者の身体に及ぼす影響についての報告は，わが国のみならず海外においても極めて少ない。本研究によって

年齢による影響の違いも明らかになるため，有意義なものと考えた。

２．研究の目的

われわれは今まで，小児の身体的虐待に基づく腎臓内への hemoglobin 沈着や頭部外傷に基づく軸索，膠細胞などの変化を免疫組織化学を用いて明らかにしてきた。これらの免疫組織化学的研究をさらに発展させて，小児・配偶者・高齢者に対する虐待の法医病理学的証明，虐待の程度と期間の推定，身体機能に及ぼす影響などの事項を究明するのが本研究の目的である。

得られた成果を総合して，より精度の高い死因推定法や虐待の法医病理学的証明法，虐待の期間や程度の推定法を明らかにして法医実務に貢献し，さらに虐待が各臓器の機能に悪影響を及ぼすことを社会に向けて広く発信していき，ひいては虐待予防に役立てていくのが本研究の最終目的である。

３．研究の方法

（１）虐待に基づく胸腺・副腎の変化に関する研究：小児虐待に基づいて胸腺の退縮や副腎の変化が認められることは知られているが，高齢者虐待の場合にも変化が見られるかを検討して，虐待の法医病理学的証明法として有用か否かを明らかにした。すなわち，高齢者虐待例の胸腺や副腎の重量，損傷や虐待期間との関係について対照例と比較した。併せて，これら臓器の組織病理学的所見についても比較した。

（２）虐待に基づく各臓器への白血球浸潤に関する研究：外傷性・出血性ショックの際に白血球が各臓器に浸潤することが知られている。高齢者への身体的虐待に基づく広範かつ新旧混在する外傷によっても同様の白血球浸潤が認められるか否かを検討し，虐待の法医病理学的証明法として有用か否かを明らかにした。白血球浸潤の程度については myeloperoxidase (MPO)を白血球のマーカーとして検討した。

４．研究成果

（１）高齢者虐待に基づく胸腺・副腎の変化
(Thymus and adrenal gland in elder abuse)

① Abstract

Endogenous glucocorticoid-induced thymic involution is generally considered to be an important finding for determining child abuse. The present study investigated the weight of the thymus and the adrenal glands in elder abuse cases to identify a potential marker for elder abuse. There was no significant difference in the thymus and the adrenal weight between elder abuse and control cases. However, the elder abuse cases in which the duration of abuse was less than 3 months showed a significant increase in the adrenal weight in comparison to control cases. In such cases, histopathological findings showed a loss of intracellular light granules from the zona fasciculata, which might indicate a loss of cholesterol due to the overproduction of glucocorticoid. These results might imply that the elderly, who were maltreated for less than 3 months, were in the early phase of a chronic stress state during which stress-induced overproduction of glucocorticoid was observed in adrenal glands as indicated by Selye. Our results suggest that increase in adrenal weight may be a potential marker for elder abuse of relatively short periods, especially less than a few months.

② Introduction

Recently, abuse and neglect of the elderly have been recognized as a widespread and growing problem. In the United States, between 1 and 2 million elderly persons (the age of 65 and over) are maltreated each year. Also in Japan, the incidence of elder abuse may also be increasing because of the country ' s rapid demographic shift to an aging society.

Thymic involution, as defined a dramatic loss in thymic weight and volume coupled to an insufficient functioning of the thymus and reduction of thymopoiesis, is generally considered to be an important finding for determining child abuse. The thymus is the organ most sensitive to stress, and stress due to maltreatment causes thymic involution. In most adult patients, it is well known that age-associated thymic fatty replacement and decrease of the thymic epithelial space size, in which thymocytes present and thymopoiesis occurs are observed. However, several lines of accumulating evidence have demonstrated that the remaining thymic epithelial space in adults were also involuted by stress in several pathological states.

In addition, the adrenal glands are also a stress-related organ and play an important role in adaptation to various

forms of stress. Further, the pathophysiology of adrenal glands is known to be changed by hormonal and nervous system stimulation during a stress state. In autopsy cases of child abuse, histopathological findings such as thickening of the capsule and lipid depletion in the zona fasciculata are known to be observed in the adrenal glands.

Therefore, the weight and histopathological findings of the thymus and adrenal glands were investigated in abused elders and the possibility of a potential marker for elder abuse was explored in the present study.

③ Materials and Methods

Twenty-three cases of elder abuse (65 years and over of age) and 29 control elder cases without any history of abuse were collected from the Department of Legal Medicine, Graduate school of Medical and Dental Sciences, Kagoshima University (4 abuse cases, 12 control cases) and the Department of Legal Medicine, Gifu University Graduate School of Medicine (19 abuse cases, 17 control cases). The elder abuse was carefully determined based on the police reports and autopsy findings. The control cases included victims of traffic accidents (13 cases), exsanguination (13 cases) and ischemic heart disease (3 cases). In each case, the victim's details including age, gender, length, body weight, the thymic weight, the adrenal weights and cause of death were reviewed. In addition to them, type of abuse, duration of abuse, degree of the physical abuse and who is assailant were investigated in abuse cases. The weight of the thymus and the collective weight of both adrenal glands were compared between the abuse and control cases. Moreover, the relationship between the duration of abuse and the thymus or adrenal weights was investigated in cases in which the duration of abuse could be estimated by postmortem findings and police reports. Cases in which hemorrhages were detected in either the thymus or adrenal glands were excluded from each study. Formalin-fixed, paraffin-embedded sections of the thymus and adrenal glands from several cases were stained with hematoxylin and eosin (H-E) for the histopathological analysis.

The means and standard error of the means (SEMs) were calculated for all parameters determined in this study. The statistical significance was evaluated by using a one-way analysis of variance (ANOVA) followed by Tukey-Kramer's method as a post-hoc test. A value of $P<0.05$ was considered to indicate a significant difference.

④ Results

Thymus: There was no significant difference in the thymus weight and in the both relative thymus weights (thymus/body weight and thymus/length) between the elder abuse and control cases. The elderly with a history of maltreatment for less than 3 months showed a tendency towards a decrease in the thymus weight and in the relative thymus weights in comparison to control cases, but the differences were not significant. Histopathological analysis revealed a massive fatty replacement in the thymus of all elder cases. In abuse cases, slight decrease in the number of thymocytes was observed, but there were no apparent differences in the number of the thymocytes between the abuse and control cases. Moreover, a starry sky appearance due to phagocytosis of apoptotic thymocytes by macrophages was not observed in abuse cases.

Adrenal gland: There was also no significant difference in the adrenal weight and in the both relative adrenal weights (adrenal glands/body weight and adrenal glands/length) between the elder abuse and control cases. However, the elder abuse cases with a history of maltreatment for less than 3 months (duration of maltreatment: several hours to 2-3 months) showed a significant increase in the adrenal weight and in the relative adrenal weights in comparison to control cases (adrenal weights, 14.000 ± 2.064 vs. 10.239 ± 0.517, $P<0.05$; adrenal weights/body weight, 0.350 ± 0.059 vs. 0.214 ± 0.011, $P<0.01$; adrenal weights/height, 0.090 ± 0.011 vs. 0.067 ± 0.003, $P<0.05$; Fig. 1). In contrast, the elder abuse cases with a history of maltreatment for more than 6 months failed to show significant changes in the adrenal weights in comparison to control cases. Microscopically, adrenal glands in the elderly maltreated for several hours and several days showed a numerous acidophilic cells with a loss of intracellular light granules in the zona fasciculata in

comparison to the control case.

Fig. 1 The relationship between the adrenal weight and the duration of elder abuse. $^{**}P<0.01$, control vs. <3m.

⑤ Discussion

Thymic involution is well known to be found in child abuse cases. In general, many kinds of stress to the body affect the hypothalamo-pituitary-adrenal (HPA) axis and the autonomic nervous system, via the release of glucocorticoids and catecholamines, respectively. In child abuse, an increase of endogenous glucocorticoids by the stress due to maltreatment has been thought to induce apoptosis of T lymphocytes in the thymic cortex, resulting in thymic involution. Although the diagnostic markers for child abuse based on the thymic involution have been extensively studied, those for elder abuse have not been investigated.

In the present study, there was no significant difference in the thymus weight and in the relative thymus weights between the elder abuse and control cases, although it has been demonstrated that the thymus in adults were also involuted by stress. In our study, histopathological analysis in the thymus showed a massive fatty replacement and remarkable decrease of thymic epithelial space in both abuse and control cases. Although a starry sky appearance, characteristic of mild stress involution was not observed, slight decrease in the number of thymocytes was observed in abuse cases. Accordingly, a slight decrease in the number of thymocytes in elder abuse cases observed in our study may not lead to weight loss of the thymus. Thus, age-associated thymic fatty replacement might mask the thymic weight loss by maltreatment in such cases. These results suggest that the thymus weight is not useful as a universal diagnostic marker for elder abuse.

As for the adrenal gland, the elderly individuals with a history of maltreatment for less than 3 months showed a significant increase in the adrenal and in the relative adrenal weights in comparison to the control cases, although there was no significant difference in weights between the total abuse and control cases. In addition, histopathological findings in such cases showed a loss of intracellular light granules from the zona fasciculata due to overproduction of glucocorticoid, which might indicate a loss of cholesterol, a material for glucocorticoid biosynthesis. Glucocorticoid secreted from adrenal glands in response to various physical and mental forms of stress. According to Selye's stress theory and recent investigators, adrenal glands are enlarged as a reflection of the overproduction of glucocorticoid (cortical hypertrophy) in the early phase of a chronic stress state which Selye called the "alarming reaction stage (A-R)". In the latter phase of a chronic stress state which Selye called the "resistance stage", production of glucocorticoid is reduced to the lowest level for resisting the stress, so that adrenal glands return to normal. Accordingly, a history of maltreatment for less than 3 months might correspond to the A-R of chronic stress as indicated by Selye. The tendency of thymic weight loss in such cases might be the consequence of thymic involution by hyperglucocorticoidemia during the A-R. On the other hand, the elderly with a history of maltreatment for more than 6 months showed similar adrenal weight as control cases, because they might be in the resistance stage as indicated by Selye. Although we could not mention about the elders with a history of maltreatment for 3 to 6 months, our results suggest that the increase in adrenal weight might imply elder abuse with a history of maltreatment for relatively short periods. It remains to be proved since when the adrenal enlargement occurs in response to chronic stress. Selye reported that the histopathological change of A-R was observed in rats with stress for 48 hours. Our results showed that enlargement of the adrenal gland was observed in a case of elder abuse with a history of maltreatment for several hours. Therefore, it is

possible that the adrenal enlargement occurs from several hours after maltreatment.

In conclusion, the thymus weight is not useful as a universal diagnostic marker for elder abuse. In contrast, adrenal weights in elder abuse cases with a history of maltreatment for less than 3 months significantly increased in comparison to control cases. In addition, histopathological findings showed a loss of intracellular light granules from the zona fasciculata, which might indicate a loss of cholesterol due to the overproduction of glucocorticoid in those cases. Our results suggest that increase in adrenal weight coupled to a loss of intracellular light granules from adrenal cortex in histopathological analysis might be a potential marker for elder abuse with a history of maltreatment for relatively short periods, especially less than a few months.

（2）小児・高齢者虐待死の諸臓器における好中球浸潤の検討

①緒言

近年，小児・高齢者に対する虐待が社会問題となっており，法医解剖においても虐待被害者の死亡例を少なからず経験する。虐待予防という社会的意義からも虐待の正確な診断は法医実務において必要不可欠であるが，非常に困難な場合があるというのが現状である。

ところで，広範な外傷及びそれに続発した外傷性ショックや出血性ショックでは，種々の臓器に好中球が浸潤し，炎症性サイトカイン，好中球エラスターゼ，ロイコトリエン，一酸化窒素などの炎症性メディエーターを産生することで臓器障害をもたらすことが知られている。そこで，今回，小児・高齢者虐待死例の諸臓器における好中球の出現を免疫組織学的に評価し，虐待の法医病理学的診断の指標となり得るか否かを検討した。

②材料及び方法

試料は，当教室で行われた法医解剖例のうち死後経過時間が 4 日以内のものを対象とし，小児剖検例 15 例，高齢者剖検例 14 例を選択した。小児剖検例は，虐待死 5 例，対照例として交通外傷 4 例，失血 2 例，その他 4 例の 4 群を検討した。また，高齢者剖検例は，虐待死 4 例，交通外傷 4 例，失血 3 例，墜転落 3 例の 4 群を検討した。小児・高齢者ともに明らかな感染が臓器に認められる例は除外した。

各症例の心臓（左室後壁），肺（左肺上葉），肝臓（右葉），腎臓（左）のホルマリン固定後パラフィン包埋切片を作製し，好中球に対する抗 myeloperoxidase 抗体を 1 次抗体として免疫染色を行った。染色した組織標本を光学顕微鏡下で 10 視野観察し，MPO 陽性細胞数すなわち好中球数の平均値を求め，各群で比較検討した。統計学的解析は，one-factor ANOVA を用いて 4 群間の比較を行い，post-hoc test として Tukey-Kramer を用いて，各 2 群間の比較を行った。有意水準 5%未満を有意な差とした。

③結果

小児対照例における各臓器の MPO 免疫染色標本では，心臓，腎臓の MPO 陽性細胞は 1 視野あたり 2，3 個程度であり，肺，肝臓では 10～20 個観察された。一方，虐待死例では，MPO 陽性細胞数は，心臓，肺，肝臓，腎臓のいずれの臓器においても他の群と比較して明らかに有意に高値を示し，肺，肝臓では特に多数認められた（図 1）。

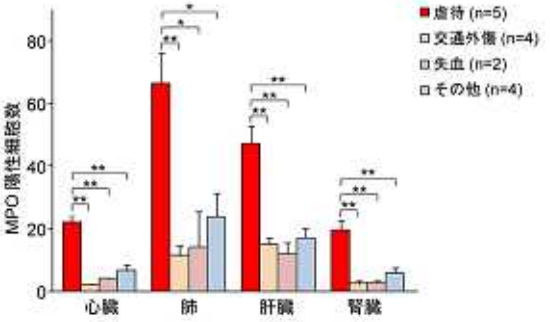

図 1．小児剖検例各臓器の MPO 陽性細胞数

高齢者剖検例では，対照例である交通外傷例，失血例，墜転落例はいずれもほぼ同様の結果であり，心臓，腎臓では 1 視野あたり MPO 陽性細胞は 2，3 個程度，肺，肝臓では 20 個前後観察された。一方，虐待死例では，MPO 陽性細胞数は，肺，肝臓で他の群と比較して有意に高値を示した（図 2）。

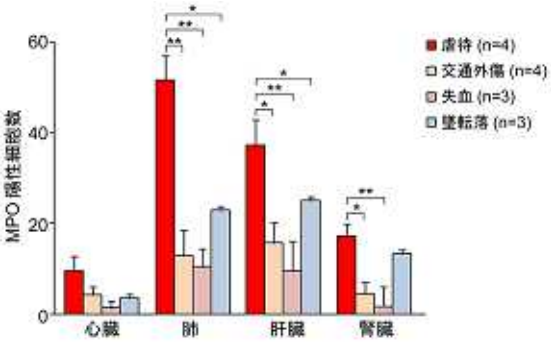

図 2．高齢者剖検例各臓器の MPO 陽性細胞数

④考察

MPO 免疫染色による諸臓器，特に肺，肝臓の陽性細胞数は虐待死例で有意に高値を示したことから，小児・高齢者虐待の法医病理学的診断の指標となることが示唆された。諸臓器のうち，肺や肝臓といった肺胞マクロファージ，クッパー細胞などの組織常在性マクロファージが存在する臓器に好中球の出現率が高値を示したことから，好中球浸潤へのマクロファージの関与が考えられた。今後，マクロファージに対する検索に加えて，諸臓器への好中球浸潤に関わる接着分子やケモカインの諸臓器における発現を検索することで，より総合的な虐待の法医病理学的診断法の確立を目指したいと考える。

５．主な発表論文等
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６．研究組織

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