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Change in Circulating Immune Complex and Its Clinical Significance in Malignancy of Gastrointestinal Tract and Liver

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SUMMARY : Forty-seven patients with digestive tract malignancy and some benign diseases (cholelithiasis, duodenal ulcer) disease were subject to this study to clarify the changes in immunologic state in terms of complements. It has been often reported that circulating immune complex (CIC) was detected in infections disease. But CIC is also able to be detected in malignant disease without infection. The blood level of CIC changes regularly in perioperative period at the time of surgical resection of malignancy. In fact, preoperative value returned to normal in absolute curative operation for gastric cancer. Meanwhile, it was variable during 7 days after operation in either benign or malignant diseases of gastrointestinal tract. The reason is that early period of surgery tends to be affected by operation insult, induced catabolic metabolism, nutritional defect and infection. After surgery to the liver, the changes in CIC were characteristic and CRA was suppressed coutinuously. Complement protein C_3, which is the most important component in CRA reaction, is generated in the liver. Therefore, it is considered that blood level of CRA is easily affected when hepatic surgery is made.

INTRODUCTION

Immunological depression of the host to surgical stress may be one of the most important problem to improve the outcome of surgery. There are many studies on cellular immunity in association with operation, but it is very rare on CIC and CRA. Serum level of CIC is often measured in order to assess the curability of collagen diseases. And it also rises in infectious diseases. But its value may be utilized in assessment of severity of malignant disease which is not complicated with collagen disease and has no infection. Kusakawa[7] proved high level of CIC in advanced cancer in 1983. While CRA was able to assay without radioisotope in 1980 by Takahashi[5]. He has proved that complement protein C3 was the most important element in reaction of CRA. Nishikawa[8] reported that C_3 increased as developement of malignant disease in 1976. So, CRA may also change due to severity of cancer or surgical intervension. Then, it is of value to clarify the immune mechanism to know the changes in CIC and CRA in relation to surgery and advancing the cancer stages. The purpose of this study is to clarify their postoperative changes and clinical significances.

MATERIAL AND METHOD

Forty-seven patients with gastrointestinal cancer, hepatoma and some benign diseases were eligible for this study. There were 24 men and 23 women. The mean age was 60.8 years, with a range of 26-79 years. CIC was measured by polyethylene glycol complement consumption

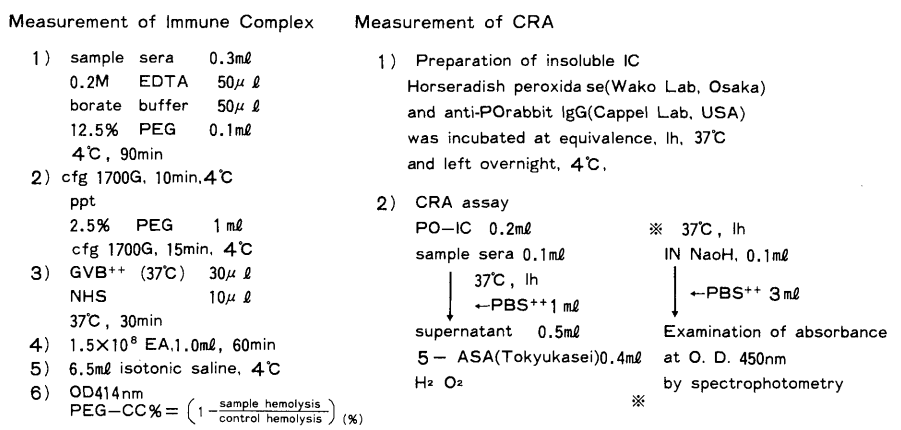

Fig. 1.

test. CRA was measured by AMANO's method. Nutritional status was assesed by weight, arm circumference (AC), arm muscle circumference (AMC), triceps skinfold thickness (TSF) and rapid turnover protein which was retinol binding protein (RBP), prealbumin (PA) and transferine (TF). The measurement of CIC was as follows.

Borate buffer, EDTA and PEG 6000 were mixed properly. It was kept at 4℃ for 90 min, centrifuged at 1700 G for 10 min and washed by 2.5% PEG, followed by spun again at 1700 G for 15 min at 4℃. The pellect was added by GVB^{2+} and NHS, incubated at 37℃ for 30 min. It was maintained at 37℃ for 60 min after the addition of EA, and was mixed with cold isotonic saline. The absorbance was measured at O.D. 450nm by spectrophotometry. The measurement of CRA was as follows. Precipitable immune complex produced by horseradish peroxidase and anti-PO rabbit IgG for an hour at 37℃ was left overnight at 4℃, followed by wash out by ASA. PO-IC and sample sera incubated and centrifuged. After addition of ASA, it was incubated at 37℃, The reaction was discontinued by NaOH. The value of absorbance at O.D. 450nm were regarded as the value of CRA. (Fig. 1)

RESULT

The two patients with elevated CIC in 6 benign diseases were complicated with infectious diseases. Blood level of CIC in the presence of malignant disease rose more than that in normal adult. But one with a large size hepatoma had minimal value of CIC. Its value in all early gastric cancers and colon cancers were almost within normal limit. (Fig. 2)

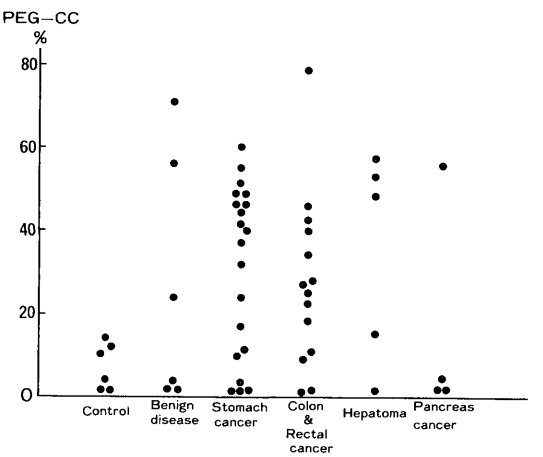

Fig. 2. PEG-CC% of peripheral blood tended to increase in malignancy. Benign disease with infection had higher CIC value

CRA values of stomach cancers increased in comparison with the control. ($p<0.01$) It was also statistically significant in colon and rectal cancers ($p<0.05$). But in hepatoma CRA levels were lowered when compared with those in stomach cancers. ($p<0.05$) In benign disease and pancreas cancers, there were no statistical significance. In hepatoma CIC levels were reduced, and it was characteristic in compari-

son with those in other gastrointestinal malignant diseases. (Fig. 3)

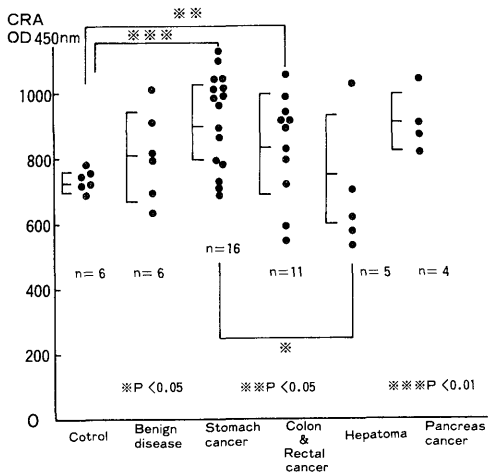

Fig. 3. The CRA value of gastrointestinal cance r increased in comparison with control. But its value of hepatoma lower than that of stomach cancer.

It was disclosed that CRA changed according to the disease stage in gastric cancers. The values in stage II diseases increased more than those of the control. ($p<0.05$) An increase in CRA in stage IV was evident and statistically significant. ($p<0.05$). The CRA levels increased in stage III. ($p<0.01$) In early gastric cancers the CIC and CRA levels were almost normal and there was no difference from the control. (Fig. 4)

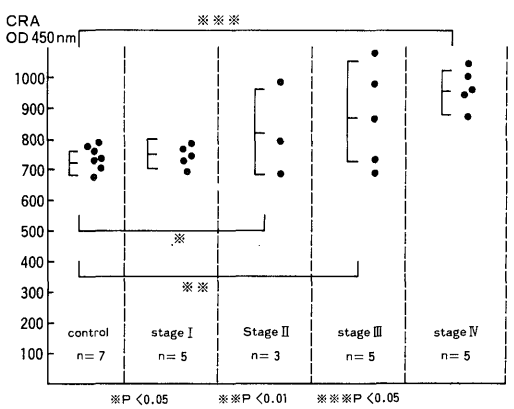

Fig. 4. The CRA value of stage I gastric cancer was unchanged. But its values of stage II and IV increased comparatively.

CIC was measured periodically in six patients with benign diseases. There were 3 cholelithiasis without jaundice, 2 duodenal ulcers and 1 ulcer of ileum. In 2 cases CIC was elevated, while it returned to normal soon after operation. In 4 cases CIC varied with a varying variety until on day 14 after surgery. Such a tendency was remarkable from the 3rd to the 10th day after surgery in 4 cases. (Fig. 5)

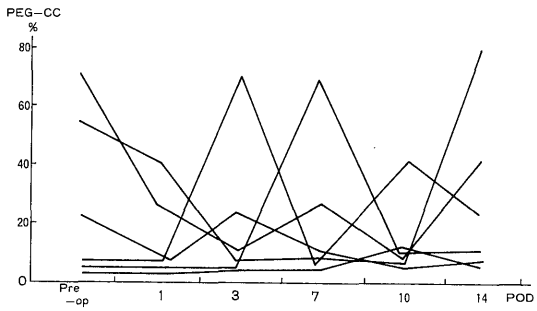

Fig. 5. Peripheral blood level of CIC was measured periodically after operation of benign diseases. It revealed variable change from 3rd day 10th day.

There were 5 cases whose CIC were not detected or less than 10%. Three were cholelithiasis, others were duodenal ulcers and early gastric cancer. Each case revealed variable changes from the 3rd to 10th day, but all recovered to preoperative level on the 14th day after surgery. (Fig. 6)

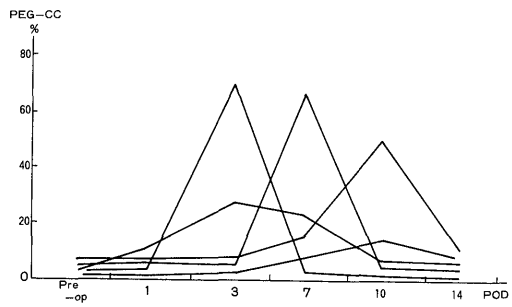

Fig. 6. There was 5 cases whose CIC level was not detected or less than 10% in preoperative day. Each case had variable CIC level from 3rd day to 10th day. All cases were recovered to preoperative level on 14th day.

Postoperative changes of CIC in gastric cancer in relation to oncological curability were evaluated. Closed circle means absolute

curative resection cases, and open circle means noncurative resection cases. Preoperative CIC levels had increased except for 2 cases. It tended to be lowered until the 3rd day. All revealed variable changes from the 3rd to the 10th day. It was reduced on the 14th day in absolute curative cases, and was statistically significant. ($p<0.05$) But there was a tendency toward an increase in patients with non-curative resection. (Fig. 7)

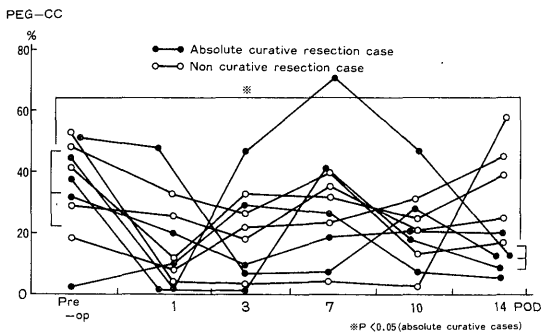

Fig. 7. These CIC values showed postoperative change of gastric cancer in relation to its curability. It was decreased on 14th day in absolute curative cases. Some case had higher CIC level on 14th day than preoperative level in non-ctrative resection cases.

Five hepatocellular carcinomas were studied. Two showed a normal CIC and another had an elevated CIC in the perioperative state. Right lobectomy in 2 left lobectomy in 1, S_2S_3 resection in 1 and S_7S_8 resection in 1 were performed. CIC levels in these patients who underwent hepatectomy for hepatoma were depressed until the 7th day. These increased markedly in 3 cases on the 7th day or the 10th day. But they returned to the preoperative level on the 14th day. The postoperative courses were different from those in benign or other malignant diseases of the digestive tract. (Fig. 8)

Changes in CRA were evaluated in 5 cases. The CRA levels in all were continuously low for 14 days. (Fig. 9)

In patients with hepatectomy and gastrectomy, the nutritional status was studied.

There is no statistical significance in anthrophemetic measurement (Weight, AC, AMC, TSF) between the patients with resec-

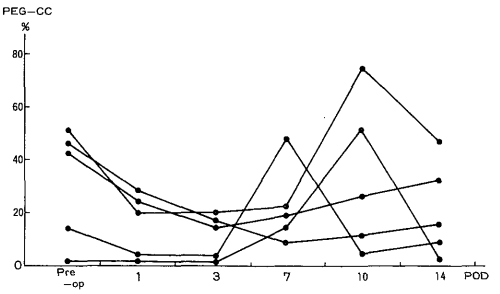

Fig. 8. 5 hepatoma were studied. There were 2 right lobectomy, a left lobectomy and 2 resection of Counaud's Segment. PEG-CC% of all were depressed until 7th day.

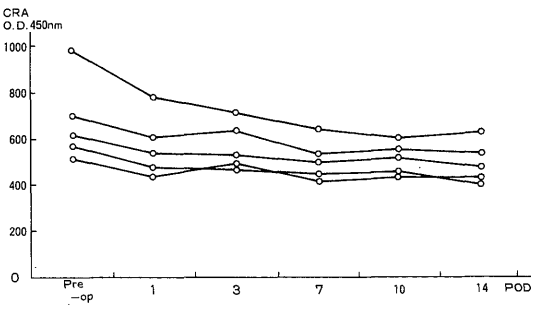

Fig. 9. Complex release activity of complement were studied in 5 hepatoma. CRA value of all case wene continuously depressed during 14 days after operation.

tions of the liver and the stomach. (Fig. 10)

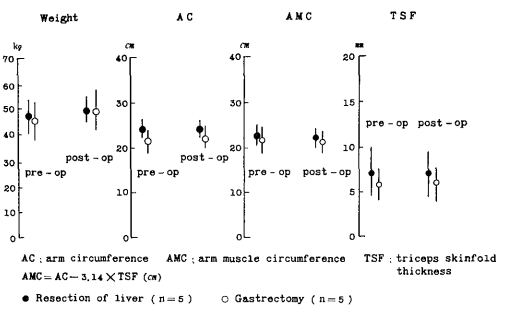

Fig. 10

Retinol-binding protein recovered on the 7th day in gastrectomy ($p<0.05$), but by partial resection of the liver it rose to normal on the 14th day ($p<0.01$). (Fig. 11)

The postoperative levels of prealbumin showed no remarkable change for 14 days both the patients with the resection of the liver and

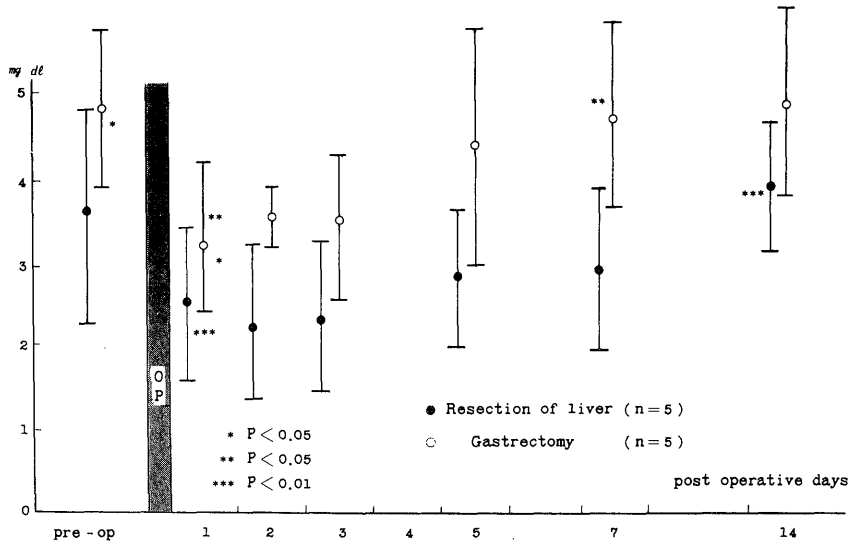

Fig. 11

the stomach. (Fig. 12)

The transferine level on the 3rd day was much more elevated than its level on the 1st day in gastrectomy. ($p<0.05$) No remarkable change was found for 14 days in patients with partial resection of the liver. (Fig. 13)

DISCUSSION

HARKISS and BRAUN[1] reported a new assay for the detection of CIC, the polyethylene glycol precipitation complement consumption assay (PEG-CC) in 1979. Since that time, the method of CIC detection has become common in the clinical use. However, a method according to Raji cell assay[2] is unavoidable with the use of isotope so that it is a great barrier to use in the laboratory. On the other hand, PEG-CC measurement require a spectrophotometric method. Therefore PEG-CC is available with

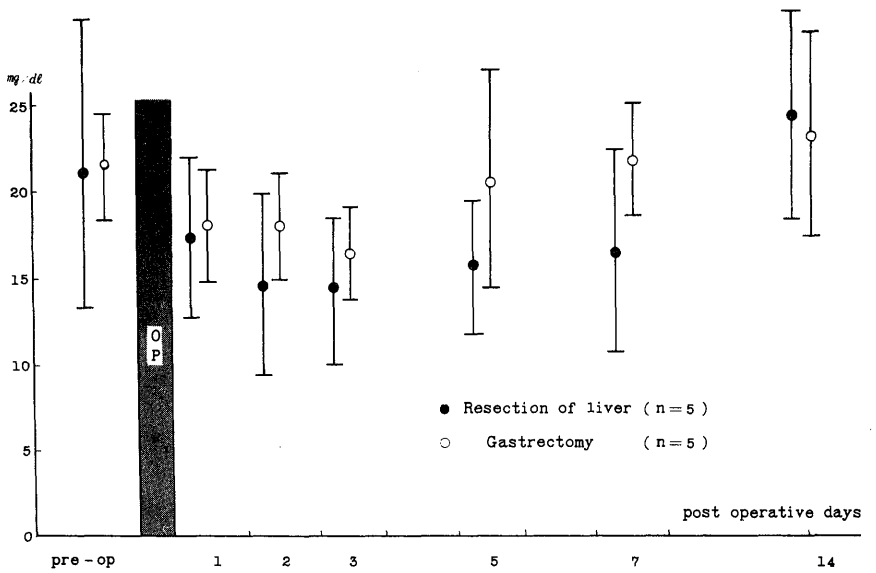

Fig. 12

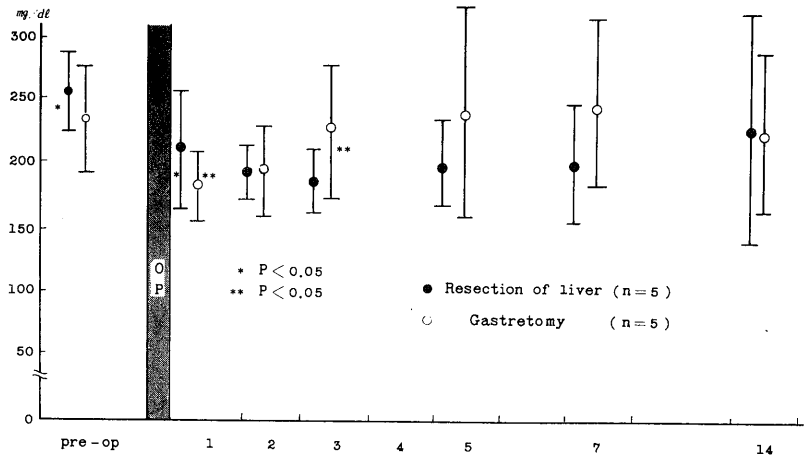

Fig. 13

the detection of CIC. The CIC values measured by PEG-CC are not different from those obtained by Raji cell assay. There were a few reports with respect to the detection of CIC in malignant diseases[3]. Each author reported that CIC increased according to the extension of cancer infiltration. Detected IC by PEG-CC did not show a special pattern in any malignant and infectious disease. There are some trials to evaluate the changes in IC for specific[4] tumor antigens, although it was not commonly employed and not standardized. PEG-CC was a kind of non-special IC. It was often detected in malignant diseases without infection. In fact, it was assumed that CIC was consistent with staging of a malignant disease. The changes in CIC also well reflects the effectiveness of surgical treatment and the adequacy of chemotherapy administered. Immune complex is metabolized chiefly in the liver. It was thought that changes in CIC was specialized when the liver underwent surgical intervension. One of the purpose of this study is to clarify the mechanism about changes in CIC. On the other hand, TAKAHASHI[5] first reported the result of the measurement of CRA by the use of radioisotope. Recently it became clear that CRA can be measured by spectrophotometry[6]. The results by this method are not different from those by RI method in accuracy. This method was commonly used and applied to monitoring collagen and infections diseases to assess the effect of therapy. It is of great use that positive CRA is indicative of the presence of malignant diseases. There were variable changes in the present study of benign diseases with infection, such as cholecystitis and pneumonia. Meanwhile, CIC levels changed in regular manner as far as gastric cancer was concerned. When the cancer lesion was curatively resected, its level significantly fell down until the 14th day. On the contrary, it remained invariable in patients with non-curative operation. In addition, a different mode of CIC change was found in hepatic surgery as compared with in other gastrointestinal surgeries for carcinomas. The blood levels of CIC were often suppressed for 10 days after operation. It seems that surgical intervension to the liver contributes to the reduction of CIC levels, which is a reflection of IC metabolism.[7] The generation of IC was also inhibited on the condition of surgical damage to the liver, although changes in IC were free from surgical insult on the 14th day.

The alternation of CRA means the complement activity in alternative pathway. It is widely accepted that complement proteins, for example C_3 or C_4, increased according to the development of carcinoma[8]. CRA fails to indicate the amount of comlement protein. CRA reflects an activity of alternative pathway of complement. The initial reactant of the pathway was C_3. C_3 was produced in the liver. Consequently it may cause depression of CRA by hepatic surgery. In fact, CRA was deppressed

in evolving hepatoma much more than in other malignant diseases. An appearance of CRA in blood also correlates with an advance in gastric cancer extension. However, study on CRA has been sporadically reported. It was also clarified in this study that functional activity of complement, that is CRA, increased according to advance of the disease stage of gastric cancer. On the other hand, CRA in hepatoma was deppressed much more than in other malignant diseases, It is important to contemplate a mechanism about CRA change. Since liver function seems to contribute to maintain CRA value, CIC and CRA changes revealed a specifical pattern in hepatoma. Low blood levels and inactivated complements are more likely to be indicative of great consumption as reported by TANIUCHI[9]. Based on this fact, hepatic surgery results in lowered CIC levels so that complement protein may be markedly decreased. CRA value was thought to be low to consume complement during 7 days following operation. Application of evaluation of CEA, α-Fetoprotein, CA 19-9, and other proteins detected by tumor specific monoclonal antibody and the their combination assay have been clinically used for detection of malignant diseases. It is difficult to conclude that CIC is of great benefit to detect a presence of malignant diseases and assess in the clinical course of the extension of malignant diseases. CIC and CRA changes are associated with complement consumption. It is of benefit to know coexisting infectious state or postoperative complications by evaluating these changes. Even though CIC levels were within normal or not detected, its change might has varied for 7 days after operation. On the other hand, it is important to measure nutritional index for considering the clinical significance of blood level of CRA and CIC. In fact, the first reactant of CRA is C_3. In addition, it is generated in the liver which is the most important metabolic organ. Furthermore, it is clear that CIC is mainly metabolized in the liver.

It is expected that the nutritional state is influencing on CRA and CIC values through the metabolism of the liver. In relation to nutritional index in this study, it seems to be considered that postoperative nutritionnal change is not concerned with the opetation insults of gastrectomy and hepatectomy. The fact would indicate RBP recovery is so delayed in patients with hepatic surgery related to the liver compared with those with gastrectomy that it suggests postoperative changes in CIC and CRA is more likely to be influenced by liver dysfunction. Needles to say, it must take it into consideration that CIC levels are directly influenced by factors of operative injury, catabolic metabolism, nutritional deficiency and accompanying infection in all the cases for about 7 days after operation irrespective of preoperative CIC levels.

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