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Original Article

Genetic Polymorphisms of Glutathione S-Transferase Genes and Risk of Colorectal Cancer in the Saudi Population

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الاشكال المتعددة لجين الجلوتاثيون س – ترانسفيريز ومخاطر الإصابة بسرطان قولون المستقيم في السعوديين

د. محمد طيب[1]، د. أحمد بابلغيث[1]، د. أحمد فوزي[2]، د. سليمان جستنية[3]، د. نداء بوقري[1]
قسم الوراثة الطبية – كلية الطب – جامعة أم القرى – مكة المكرمة – المملكة العربية السعودية [1]، قسم الوراثة الجزيئية – المركز القومي للبحوث – الدقي – الجيزة[2] ، قسم الجراحة – كلية الطب – جامعة أم القرى – مكة المكرمة – المملكة العربية السعودية[3].

الملخص

سرطان القولون والمستقيم من أكثر أنواع السرطان انتشارا لدى المجتمع السعودي، فعدد الحالات المسجلة سنويا يبلغ 1000 حالة تقريبا، وهو يعتبر أكثر أنواع السرطان انتشارا لدى الرجل السعودي، وثالث أنواع السرطان انتشارا لدى المرأة السعودية.

تمثل الجلوتاثيون الرابطة بجموعة الكبريت مجموعة كبيرة من الانزيمات المتشابهة والتى تعمل على التخلص من سمية كثيرمن المواد الكيمائية الضارة التى يتعرض لها الانسان خلال حياته سواء من داخل الجسم البشرى او من البيئة المحيطة.

فهناك حالة جينية (عدم ظهور النمط الجيني) لهذا الانزيم تجعله غير فعال و غير قادر على اداء وظيفته و يعتقد ان وجود مثل هدة الحالة الجينية تجعل الانسان اكثر عرضة لاصابة بانواع مختلفة من الاورام السرطانية.

ولقد اجريت دراسات عديدة للوقف على طبيعة الارتباط بين هذة الحالة الجينية التى ينتج عنها انزيم عديم الفاعلية و خطورة الاصابة بسرطان القولون والمستقيم ولكن مجمل هذة الدراسات متناقض في نتائجها على طبيعة هذا الارتباط و من اجل ذلك فقد قمنا بهذة الدراسة للوقوف على حقيقة هذا الامر في المملكة العربية السعودية بمنطقة مكة المكرمة.

وقد شملت هذه الدراسة 170 شخصاً مصاباً بسرطان القولون والمستقيم ، وكذلك 170 شخصاً اخر سليماً . وتم تحديد الحالات الجينية المختلفة بواسطة تقنية التفاعل البلمرة المتسلسل المعقد.

هذه الدراسة لم تظهرارتفاع ملحوظ بفارق إحصائي في معدل الحالة الجينية للانزيم الجلوتاثيون الرابط بمجموعة الكبريت من انواع م١ و ت١ في مرضى سرطان القولون والمستقيم قياساً بالمجموعة الضابطة . و بناءا على معطيات ونتائج الدراسة تم استنتاج عدم وجود علاقة بين تباين الحالات الجينية (ظهور النمط الجيني او عدم ظهور النمط الجيني) للانزيم الجلوتاثيون الرابط بجموعة الكبريت من انواع م١ و ت١و خطورة الاصابة بسرطان القولون والمستقيم للمرضى السعوديين في المملكة العربية السعودية بمنطقة مكة المكرمة. وعلى الرغم من هذة النتيجة فانه لا يمكننا اهمال دور هذة الجينات بالكلية فى عملية تطور وظهور اعراض سرطان القولون والمستقيم فقد يوجد تفاعل جينى لهذة الجينات موضع الدراسة مع جينات اخرى قد يودى هذا الى ظهور هذا الارتباط بسرطان القولون والمستقيم. وايضا فانه يوجد حاجة للقيام باعداد كثيرة من الدراسات على نفس النمط فى باقى بقاع المملكة لوقف على حقيقة هذة النتائج و هذا الارتباط من عدمه. وايضا مع الاخذ فى الاعتبار العوامل البيئية الاخرى و نمط الحياة اليومى وعلاقته بخطورة الاصابة بسرطان القولون والمستقيم.

ABSTRACT

Background:
Glutathione S-transferases (GSTs) are an important family of isoenzymes involved in the detoxification of many environmental carcinogens. The GSTs null deletion polymorphism of the GSTs genes that lead to diminished of enzymatic activity have been associated with increased susceptibility to develop several cancers.GSTM1 & GSTT1 status have been extensively studied as a risk factor for colorectal cancer (CRC), although inconsistent associations between GSTM1 & GSTT1 genotype and CRC risk have been observed .

Methods:
To re-examine this controversy, we have undertaken a case-control study investigating the relationship of GSTM1 & GSTT1status (null/ non-null genotype) and CRC risk, involving a total of two 170 CRC cases and individual controls. Genotyping assay was performed by multiplex PCR followed by gel electrophoresis.

Results:
The OR of CRC and GSTM1 & GSTT1 null genotype were 0.85 (0.45 – 1.59) and 1.13 (0.56 – 2.29) in the Saudi population, respectively. Hence, the results of this analysis does not support the hypothesis that either GSTM1 or GSTT1 have been associated with CRC, and suggests that GSTM1 & GSTT1 status have no effect on the risk of developing CRC .

Conclusion:
There may be interactions between GSTM1 & GSTT1and other polymorphisms that may influence the risk of developing CRC. Further investigation in different regions of the Kingdom of Saudi Arabia are required to verify or refute these results, and to identify more definite risk groups and determine factors of importance in the development of CRC.

Keywords: *GSTM1 & GSTT1*; colorectal cancer; polymorphism; genotype; KSA.

INTRODUCTION

Colorectal cancer (CRC) is considered as the fourth leading cause of cancer mortality both worldwide and in the Kingdom of Saudi Arabia (KSA). Moreover,The incidence seems to be increasing; with approximately 500,000 annual deaths (1,2). Although CRC is less frequent in the KSA than in its counterpart Gulf Cooperation Council States and in the West, this disease was the second most common malignancy after breast cancer. It ranks first among men and third among women between 1994 and 2004 (3). An increasing number of epidemiological studies

indicate that cigarette smoking; alcohol use; decrease physical activity, and the consumption of diets high in red meat are probably important etiological factors increasing the risk of developing CRC4. However, it is now widely accepted that CRC risk is determined by a complex interaction of both genetic and environmental factors such as susceptibility genes, carcinogen exposure and dietary factors (4-6).

Drug metabolizing enzymes modifies chemical compounds in cigarette smoke or diets, and some of the metabolites may be the cause of CRC. Polycyclic aromatic hydrocarbons (PAHs) and other tobacco-related carcinogens are activated by phase I enzyme cytochrome P450 that is termed high risk gene and is detoxified by phase II enzyme glutathione S-transferases (GSTs). The metabolic balance between phase I and phase II enzymes may be of importance to determine genetic susceptibility to colorectal carcinogenesis, as well as lung cancer (7-9). Following phase I reaction, phase II enzymes such as GSTs are responsible for detoxification of activated forms PAH epoxides and protect cells from reactive chemical intermediates and oxidative stress.

GSTs are constitutively found in a wide variety of tissues, with different characteristic patterns of GST isozymes. *GSTs* also form a superfamily of genes consisting of eight distinct families, termed alpha (α), mu (μ), pi (Π), theta (θ), sigma (δ), zeta (ζ), kappa (k) and gamma (γ) (http:www.OMIM.org). Certain genes within the *GSTM*, *GSTT* and *GSTP* subfamilies (*GSTM1*, *GSTT1* and *GSTPI* genes, respectively) are polymorphic in humans. GST expression varies between individuals, and expression is tissue and sex specific. *GSTM1* and *GSTT1* are expressed in normal colon tissue. The phenotypic absence of *GSTM1* and *GSTT1* activity is due to homozygosity for deletion of these genes, termed the null genotype (9, 10). Inheritance of null alleles in the *GSTM1* (chromosome 1p13.3) and *GSTT1* (chromosome 22q11.2) genes is common in the population, varies by ethnicity, and is associated with the loss of enzyme activity and cytogenetic damage. The homozygous deletion of *GSTM1* gene has been shown to occur in approximately 50% of the populations of various ethnic origins (11-17), while the homozygous deletion of *GSTT1*gene has distributed between 10 and 64 % of various ethnic groups (11-13,15).

The frequency of the *GSTT1* null genotype in Caucasian populations is 30% or less, but that in Oriental populations may be similar to the frequency of the *GSTM1* null genotype (15). In an earlier study based on phenotyping, the *GSTM1* null genotype was found to yield increased risk for CRC, {(OR) of 2.32 (95%CI: 0.88-6.15)} in English groups (8). In the next study, an excess of the individuals with null genotypes were observed in CRC but this was not a significant. When the patients were divided into cancers occurring in the proximal or distal colon, the null genotype became a significant risk factor among the subgroup with distal colorectal tumors11. Therefore, two (8,11) of eight (8,11-17) revealed approximately 2-fold increased risk for colorectal cancer.

Five published studies (11-13,15,17) have examined the relationship between *GSTT1* null genotype and colorectal cancer risk. Only one (17) of these studies showed the *GSTT1* null genotype was related to significantly increased risk CRC. It is likely

that individuals with more reactive phase I enzymes and less efficient phase II enzymes might be at higher risk for different types of cancer than individuals with the opposite combination (18-23). Recently, lots of meta-analysis and case control studies claimed that the null genotypes of *GSTM1* and *GSTT1* and the dual null genotype of *GSTM1*/*GSTT1*, were all not risk factors in CRC among the Central European (24), Chinese (25), and South Indian populations (26). Whereas, another study in same year suggested that GSTs measurement might be useful as a colorectal marker in CRC (27). The aim of this study was to explore the most real possible association between*GSTM1*& *GSTT1* status and CRC risk in Saudi population.

METHODS

Blood samples of this case-control design study were obtained from seventy Saudi patients newly diagnosed with CRC. One hundred matched controls were selected consecutively. All cases and controls were Saudis over 30 years of age and represented both sexes. The selection of controls was matched to the cases in relation to both age and sex. A case was defined as a newly-diagnosed CRC patient who is free from other chronic diseases such as diabetes, hyperlipidemia, hypertension, cardiac, liver and renal diseases. Female cases were not pregnant or lactating. Controls were free from cancer or chronic diseases. The study was approved by the ethical committee of the Medical School of Umm Al-Qura University. All patients and controls gave informed, written consent to participate in the study.

Laboratory methods

Genomic DNA was extracted and purified from EDTA-peripheral blood using the QIAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The purified genomic DNA was quantified and stored at -80°C until required. For genotyping assay, PCR was performed in which GSTM1 and GSTT1 were co-amplified using the primers M1F: (5'-GAA CTC CCT GAA AAG CTA AAG C-3') and M1R: (5'- GTT GGG CTA AAT ATA CGG TGG-3') and T1F: (5'-TTC CTT ACT GGT CCT CAC ATC TC-3') and T1R: (5'-TCA CCG GAT CAT GGC CAG CA-3'), generating fragments of 215 and 480 bp, respectively, based on a previously described method23. Length specific PCR amplifications of the primer sets used were confirmed by an in silico search of the Genome sequence using the UCSC genome browser In-Silico PCR software (28). The absence of one PCR product for one gene indicated a null genotype for this gene. The amplification of GSTM1&GSTT1 genes were carried out by mixing 50 ng of the isolated DNA, 25 ng of primers, 1X Master Mix from Thermo Scientific Inc. (Maxima Hot Start Green) containing Maxima Hot Start Taq DNA Polymerase, optimized hot start PCR buffer, Mg2+, and dNTPs. A total of 35 cycles of PCR using the DNA Engine Dyad thermal cycler from Bio-Rad laboratories Inc. with denaturation at 94oC for 30 sec, annealing at 580C for 60 sec, and extension at 72oC for 60 sec was performed. An initial denaturing was carried out at 95 oC for 4 minutes and a final extension step at 72oC for 10 minutes.

Early experiments for optimizing the annealing temperature were performed to successfully co-amplify the two target genes.

Genotypes were identified by electrophoresis of the amplified fragments through 2% agarose gels containing ethidium bromide (0.5 mg/mL). A quality control study was performed to validate the results. DNA samples were quantified by absorbance measurement and this allowing samples concentration to be normalised to produce consistent results. The genotypes for all samples were reassessed twice to confirm the results and ensure reducibility.

GSTs genes status

The following nomenclature have been used to specify the genotypes at GSTM1: non-null (wild-type (WT) or heterozygous deletion), null (homozygous deletion); and GSTT1: non-null (wild-type (WT) or heterozygous deletion), null (homozygous deletion).

Statistical analysis

The genotype and allelic frequencies for all the individuals from the CRC group were separated and compared statistically with the corresponding data for the control group. For this purpose, we used the $\chi 2$ test with SPSS 16. Results were considered to be statisti¬cally significant when the P value was less than 5% ($P < 0.05$). ORs were calcu¬lated for disease susceptibility associated with specific genotypes.

RESULTS

Genotyping data after multiplex PCR and gel electrophoresis as illustrated in figure 1 were recorded and tabulated to make it ready for statistical analysis. The frequency of the GSTM1 null genotype was 62.8% and 59.0% in CRC and control groups respectively. The frequency of the *GSTT1* null genotype was 24.3% and 27.0% in CRC and control groups respectively. The frequency of the *GSTM1*non-null genotype was 37.2% and 41.0% in CRC and control groups respectively.

The frequency of the *GSTT1* non-null genotype was 75.7% and 73.0% in CRC and control groups respectively. The frequency of the dual null genotype of *GSTM1/GSTT1*was 87.1% and 86.0% in CRC and control groups respectively.

The statistical analysis of the results demonstrated t no statistically significant association of *GSTs* genotypes with CRC cases as shown in table1. This analysis shows that the null genotypes of *GSTM1* and *GSTT1* and the dual null genotype of *GSTM1/GSTT1*were all not risk factors in CRC patients in our population.

Table1: GSTM1 and GSTT1 genotype frequencies in cases and control groups

Status	Type	CRC	OR (95%CI)	P	Control
GSTM1	Non-null null	26 (37.2 %) 44 (62.8%)	0.85 (0.45 – 1.59)	0.61	41 (41.0%) 59 (59.0 %)
GSTT1	Non-null null	53 (75.7 %) 17 (24.3 %)	1.13 (0.56 – 2.29)	0.73	73 (73.0 %) 27 (27.0 %)
GSTM1/T1	Dual null	61 (87.1%)	1.01 (0.41 – 2.12)		86 (86.0%)

Data are reported as numbers of subjects with percent in parentheses. CRC: colorectal cancer; OR= Odd

Ratio; 95% CI = confidence interval at 95%.

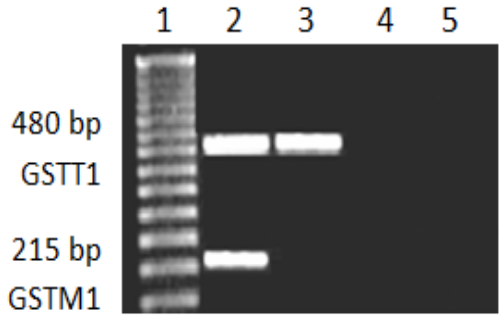

Fig.1. Example of GST genes genotypes obtained. Lane 1: DNA ladder; Lanes 2 (dual none—null) & 3 control (null *GSTM1*), Lane 4: CRC case (dual null); Lane 5: negative control

DISCUSSION

Although in this study the GSTM1 and GSTT1 status were not associated with an increased risk of CRC, the influence of the these genes in the devel¬opment of CRC cannot be excluded, since haplotypes involving many metabolic genes could contribute in a distinct manner to carcinogenesis.

The first report in 1991, evaluated a possible association between the GSTM1 and GSTT1 null genotypes and CRC (7). Since then, the GSTM1 and GSTT1 functional-loss deletion polymorphisms have been regarded as a risk factor for developing CRC by a number of researchers. It is not uncommon for the initial small size studies to over-estimate risk or effect on CRC susceptibility, which subsequent larger studies cannot confirm and thus lead to incorrect conclusions (29).

The inconsistency about the effects of GSTM1and GSTT1 null genotypes on susceptibility to CRC prompted this analysis in order to explore a possible association between GSTM1and GSTT1deficiency and CRC risk. The results of this study suggest that GSTM1and GSTT1 null genotyping is not associated with an increased risk of developing CRC.

GSTs are a superfamily of phase II isoenzymes believed to protect cells from reactive chemical intermediates and oxidative stress resulting from a wide range of electrophilic xenobiotics (e.g., tobacco-related carcinogens) and endogenous intermediates (e.g., reactive oxygen species). GSTM1and GSTM1deficiency has been evaluated as a risk factor in individual susceptibility to several cancers by a number of epidemiological studies.

Although GSTM1 and GSTT1 null genotyping did not display an association with increased risk of developing CRC in this study, it is not surprising because the evidence to support the role of GSTM1 and GSTT1 status as a colorectal cancer risk factor is not strong. It is conceivable that the influence of GSTM1 and GSTT1 genotypes may seem to be relevant to the expression levels in other tissues as GSTM1 and GSTT1 are only expressed at low levels in colon tissue (30). It is known that the primary site for the expression of GSTM1 and GSTT1 are the human liver (31). Therefore, increased CRC risk directly related to the lack of enzymes activity would be mediated by blood-borne metabolites from hepatic system.

It has been taken into consideration that the design of this case-control study in evaluating GSTs deficiency as a risk factor for CRC was far from perfect. From this data, it could be assumed that these functional-loss polymorphisms in GSTs do not alter the risk of CRC. However as with all analysis there are areas that can affect outcome and may

confound the results of analysis, giving false positives or reporting no association where there is one.

These factors have been reviewed by Cardon and Bell (32). Consideration of sample size is crucial in the design of case-control studies in order to clarify an association between genotypes and cancer risk. If GSTs deficiency is associated with 1.5-fold increase in CRC risk, this study is obviously under-powered to demonstrate such a moderate effect. Some case-control studies analyzed were based upon cancer cases and hospital based controls. The use of healthy controls is more appropriated as controls with non-malignant disease might influence the frequencies of the GSTM1and GSTT1genotypes in determining the susceptibility to cancer risk. It has been reported that hospital based controls are, in general, not desirable because they are a more or less biased population.(33)

In addition, it is now widely accepted that differences in ethnic distribution between case and control groups among the studies may be another source of a potential bias of the results from the analysis. So, the association between cancer and a particular polymorphic site in one ethnic group might be of limited value as a genetic marker for cancer in another ethnic group.

The frequencies of GSTM1 and GSTT1 deficiency varied between ethnic groups: in Caucasian populations (34). The frequency of the GSTM1 deficiency ranges from 40.8% to 58.6%; in Asian populations (11,35), from 21.6% to 55.9%; and in African populations (36), it was 66.6%. The frequency of the GSTT1 null genotype in Caucasian populations is 30% or less, but that in Oriental populations may be similar to the frequency of the GSTM1 null genotype. Ethnic specificity in this analysis would be taken into consideration during investigating the association between GSTs deficiency and CRC risk in Saudis.

As the KAS is considered as multiethnic country, affecting the outcome and may confound the results of this analysis. Depending on the control and cases, individuals may be related to different ethnic groups that may contribute to this negative association.

Glutathione S-transferases represent a complex grouping of proteins. Two entirely distinct superfamilies of enzyme have evolved that possess transferase activity (37). The first enzymes to be characterized were the cytosolic or soluble, GSTs385. To date at least 16 members of this superfamily have been identified in humans (37). On the basis of their degree of sequence identity, the soluble enzymes have been assigned to eight families (37,39,40). The second more recently defined superfamily, is composed of microsomal transferases and has been designed membrane-associated proteins in glutathione metabolism, or MAPEG for short (41).

In humans, MAPEG superfamily has at least six members. Evolution of a large number of soluble GST and MAPEG members has allowed diversification of function, regulation and subcelluler localization in the two superfamilies. GSTM1 and GSTT1 genotype is only two of key enzymes involved in the detoxification of a number of environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs). Other isoenzymesmay have a function in the

detoxification of PAHs. Thus, the lack of an association between GSTM1and GSTT1 deficiency and CRC risk may imply that GSTM1and GSTT1 genotype might have small impact on modifying CRC susceptibility.

It is conceivable that CRC risk related to any one locus will be small because gene gene interactions are likely to operate. Therefore, the effect of the GSTM1and GSTT1 genotype on susceptibility to CRC could be minor. In this study, we only addressed the question of the GSTM1 and GSTT1 status and CRC risk. We were unable to evaluate whether the presence of a gene-environment interaction differs when stratified by levels of smoking exposure, dietary and lifestyle characteristics among the studied subjects.

Such data were not available during this analysis, so any general conclusions could not be drawn based on such information. It has been reported that a sedentary lifestyle and a diet low in fruits and vegetables, and high in animal red meat and saturated fat, appeared associated with high risk of CRC (42-44). Moreover, it should be noted that some studies reported that cigarette smoking is a risk factor for CRC and fail to show an association with the GSTM1and GSTT1deficiency (45, 46). Therefore, it is likely that cigarette smoking may be an independent risk factor for CRC regardless of genotype status.

In conclusion, this study does not support the hypothesis that the GSTM1 and GSTT1 genotypes are independently associated with an increase in the risk of developing CRC. It may reflect a relatively small effect of GSTM1 and GSTT1 genotypes on CRC risk. Nevertheless, it is clear to know that the metabolism of carcinogens is complex, and interaction with other "high-risk" genes and environmental exposures may be important when assessing the risk of developing CRC.

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Original Article

Biology of Interleukin 33 (IL-33)

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العلم الحيوي لانترلوكين 33

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الملخص

انترلوكين 33(IL-33) هو عضـو جديد من عائلة محفز الخلايا انترلوكين1 (IL-1) والذي يبدي وظيفة حيوية من خلال مستقبل الخلايا الخاص به وهو ST2 .

الفكرة البدائية كانت توضــح أن انترلوكين 33 يبدي وظيفة حيوية في خلايا تي المســاعدة 2 (Th2) الحاملة لـــــ CD4 والمرتبط إيجابياً مع ST2 الذي على سطحها من خلال انتاجَ IL-5 وَ IL-13 .

من المثير للاهتمام أن الدراسات الأخيرة تقترح أن انترلوكين 33 أيضاً قد يكون مسؤولاً عن استجابة خلايا تي المساعدة 1 (Th1) في المناعة الطبيعية والحالات المرضــية . على أي حال , ســواءاً انترلوكين 33 قادراً على تحفيز خلايا تي المساعدة 1 أو لا , والذي لا يزال مجهولاً حالياً .

ABSTRACT

Interleukin-33 (IL-33) is a new member of the IL-1 cytokine family which exerts biological function via its cellular receptor ST2. The initial thought was that IL-33 exerts a vital function in ST2-positive type 2 $CD4^+$ T helper (Th2) cells response through the induction of IL-5 and IL-13. Interestingly, recent studies have suggested that IL-33 may be also involved in Th1 cell responses in immunity and disease. However, whether IL-33 can polarise Th1 cells or not is currently unknown.

Keywords: IL-33, Th1/Th2, St2 receptor

INTRODUCTION

IL-33 was discovered as a new member of the IL-1 family in 2005 (1).
The members of the IL-1 cytokine family including IL-1α, IL-1β and ……. IL-18, possess similar homological structure and nucleotide sequences and play a critical role in immunity, infection and inflammation (2-3). IL-33 is produced as a pro-protein about 32KDa which can be further matured by undefined enzymes to produce 18KDa mature protein (1).

Pro-IL-33 contains a DNA-binding domain which allows the protein to interact with chromosomal DNA in the nucleus may play a regulatory role in gene function (1). There is 55% identical homology at the amino acid level between murine and human *IL-33* (1). The mRNA level of IL-33 can widely detected in tissues such as lung, brain, stomach, spin cord and skin (1). However, the expression of IL-33 mRNA is only observed in a few cell types such as epithelial cells, smooth muscle cells (SMC), activated macrophages and dendritic cells (DC) (1).

METHODS

RECEPTOR FOR IL-33

IL-33 is thought to perform its biological function through a receptor complex consisting of ST2 and IL-1 receptor accessory protein (IL-1RAP) (1, 4). Even though IL-1RAP is necessary for signalling of IL-33, IL-33 mainly signals via ST2 (1). ST2 is a member of IL-1 receptor (IL-1R) family and is mainly on innate immune cells such as mast cells and basophils (5), eosinophils (6) and DC (7). It also preferentially induced and expressed on Th2 cells, but not Th1 cells (8-9). IL-33 also is expressed by structural cells, such as epithelial cells, endothelial cells and fibroblasts which play a major role in the immune system (10).

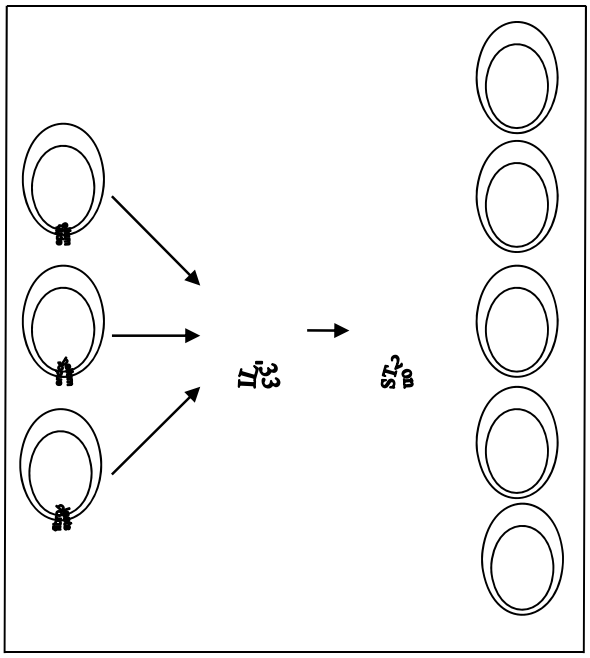

Figure1: IL-33 production and receptors

FUNCTION OF IL-33

The interaction between IL-33 and its receptors initiates the recruitment of myeloid differentiation primary-response protein 88 (MYD88) complexes to activate the transcription factor nuclear factor- kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), leading to cytokine production and cellular activation (1). It has been reported that IL-33 can drive production of Th2-secreted cytokines such as IL-5 and IL-13 but not IL-4 by either polarised Th2 cells or naïve T cells independent of IL-4 (1, 11). Furthermore, increased mRNA levels of IL-5 and IL-13 can be observed in spleen, thymus and lung by stimulation with IL-33 *in*

vivo (1). Schmitz and colleagues also reported that IL-33 can induce high level of serum IgE production, splenomegaly and eosinophilia in mice. These findings indicate that IL-33 may be a key factor for Th2 response in immunity and disease.

In addition, IL-33 can stimulate Th2-associated cytokines and protect against parasite infection and arthrosclerosis (2, 12). IL-33 also play a critical role in allergic disease and asthma due to its important function on Th2 cells, mast cells, basophils and eosinphils in allergic responses (11, 13-17).

However, several studies have revealed that IL-33 might also be involved in the Th1-mediated response (18-20). It has been reported that IL-33 can induce IFN-γ from invariant natural killer T (iNKT) cells as well as natural killer (NK) cells in the presence of IL-12 (19). It can also promote the production of pro-inflammatory cytokines such as IL-17, TNF-α and IFN-γ in mice of collagen-induced arthritis (CIA), a model for human rheumatoid arthritis (21). Thus, IL-33 can mediate Th1 cells response separately from its function in Th2 cells responses. It is also reported that IL-33 can activates the $CD8^+$ T cells and NK cells that could directly kill tumor cells. These observation show that IL-33 function like as IL-18 that can activate both Th1 or Th2 base on condition and act as a alarmins for immune system(22).

'Alarmins' are a group of endogenous proteins or molecules that are released from cells during cellular demise to alert the host innate immune system. It also activates the indirect anti-tumor immune cells such as dendritic cell (DC)(23).

Cell activation	Cytokine and Ab production	Disease
Th2	IL-5 and IL-13	Protect against parasite infection and arthrosclerosis
Th2 cells, mast cells, basophils and eosinphils	high level of serum IgE	allergic responses
$CD8^+$ T cells and NK cells	------	kill tumor cells
NK and iNKT cells in the presence of IL-12	IFN-γ	------
------	IL-17, TNF-α and IFN-γ in mice of CIA	------

Table 1: Function of IL-33

CONCLUSIONS

Cytokines play a critical role in the control of the innate and adaptive immune responses. IL-33, the most recently discovered member of the IL-1 superfamily including IL-1 and IL-18 and have been linked to several human pathologies. Il-33 strongly bind to ST2 receptor that is mainly expressed on stromal cell and Th2 cells. IL-33 has shed new light on the intricacies of immune system regulation. These novel cytokines have pleiotrophic actions ranging from antiviral immunity to the regulation of Th2 immune responses. For example, the discovery of IL-33 has significantly improved our understanding of the factors regulating the polarization of the T helper cell responses and IL-33 appears to be an important regulator of Th2 responses.

On the other hand, IL-33 considered to be critical for mounting an efficient antiviral response, which are yet to be fully characterized, are emerging as important components of the inflammatory response in allergy and autoimmunity. IL-33 and other cytokine/receptor combinations may, therefore, serve as novel targets for the

treatment and control of allergy, autoimmune diseases, and some cancers.

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Review Article

Toll-like receptors (TLRs) Review

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استعراض TLRs

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الملخص

مستقبلات شبيهة تول(TLR) هي مستقبلات يرتبط بها انماط جزيئية ذات علاقة بمسبب المرض(PAMPs) ولها دور مهم في المناعة الحامية من الإصابة بميكروب أو التهاب . وهي مكونات بدائية في جهاز المناعة الطبيعية والمكتسبة وهي فعالة كمتداخلات مركزية لنوعيات واسعة من الاستجابات , استجابة لتعكس تأثيرات لإفرازات الميكروب . تحفيز TLR بواسطة إفرازات الميكروب يؤدي إلى طرق استجابة لتنشيط ليس فقط المناعة الطبيعية ولكن المناعة المكتسبة أيضاً . روابط هذه المستقبلات TLR لها دور مهم غير مباشر في تحفيز استجابات مدعومة بخلايا تي خلال تأثيرها على خلايا المناعة الطبيعية مثل تنظيم ظهور الجسم المستضد على الخلايا واظهار الجزيء المحفز المساعد وانتاج محفز الخلايا عند الالتهاب . ليكن أكثر وضوحاً كون روابط مستقبلات TCR قادرة على التأثير المباشر على خلايا تي , ربما كجزيئات محفزة مساعدة . الدراسات على مستقبلات TLR تبدي بوضوح أهميتها في حدوث العديد من الأمراض , لتأسيس الميكانيكية المطلوبة بالضبط تحتاج تمحيص أكثر . بالعموم TLR تدعم استجابات خلايا تي الفعالة مثل انتاج محفز الخلايا والتكاثر والبقاء لخلايا تي الفعالة , في حين زيادة عدد خلايا تي المنظمة +CD4+CD25 مع فقدان مؤقت لوظيفة تثبيط المناعة . الميكانيكية الجزيئية لوظائف مدعومة بواسطة مستقبلات TLR في خلايا تي والتأثير المباشر لهذه المستقبلات على خلايا تي .

الكلمات الدالة : مستقبلات شبيهة تول – عمل الخلايا اللمفية تي - Th1/Th2

ABSTRACT

Toll-like receptors (TLRs) are pathogen-associated molecular patterns (PAMPs) recognition receptors that play important role in protective immunity against infection and inflammation. They are an essential component of the innate and adaptive immune systemans act as central integrators of a wide variety of signals, responding to diverse agonists of microbial products. Stimulation of Toll-like receptors by microbial products leads to signaling pathways that activate not only innate, but also adaptive immunity. TLR ligands indirectly play an important role in promoting T cell-mediated responses via their effects on innate immune cells including up-regulating antigen presentation, co-stimulatory molecule expression, and inflammatory cytokine production. It has also become increasingly evident that TLR ligands can also act directly on T cells, possibly as co-stimulatory molecules to modulate T cell response. Studies on TLRs clearly show their importance in induction of several diseases but establishing the exact underlying mechanism require further investigation. In general, TLRs can function as costimulatory receptor to enhance effector T responses including cytokine production, proliferation, and survival while expanding the CD4+ CD25+ Treg cell population with a transient loss of immunosuppressive function. The molecular mechanisms for the TLR mediated function in T cells and the direct effect of TLRs on T cell polarization need to be addressed.

Keywords: TLR, T Lymphocytes Function, Th1/Th2

INTRODUCTION

Protective immunity in mammal includes innate and adaptive immunity. The innate immune response is immediate, and it is the first line against pathogenic infection, but their function is limited and nonspecific. Adaptive immunity start later and is more specific and include cell mediated response by T cells and humoral response by B cells.

In contrast to innate immunity, adaptive immunity is specific for each pathogen, the response last longer because of the memory cells induction. After antigens encounter the system, the naïve T cells become activated and differentiate into T helper type 1 (Th1), Th2, TH17 or regulatory T cells based on the structure of antigen, co-stimulation and cytokine milieu. Each subsets of T cells have different function. For example, Th1 acts against intracellular pathogen but may cause inflammatory diseases. Th2 cells control against extracellular pathogens, but are also responsible for allergic responses, and there enough evidences indicate the key role TH17 cells in pathogenesis of autoimmune diseases such as RA, Psoriaris, Psoriatic arthritis, ankylosing spondylitis Therefore, It is always fascinating to an immunologist how innate and adaptive immunity is regulated. The results from these studies help us understand the etiology of some of these diseases.

The immune system has evolved primarily to combat pathogens. However, irrational exuberant of the immune response can lead to a range of autoimmune diseases. Thus, the immune system serves the mammalian hosts

in three key aspects: a. To mount an immediate defence against infection, involving innate defence (rapid but non-specific), and primary immune response (delayed but specific). B) To form a rapid and effective recall mechanism in response to re-infection(specificTandBcell memory). C)Toavoidautoimmune pathology(toleranceand regulation/suppression).

The host defence response to pathogens depends on the immune system.Adaptive immunity is a highly sophisticated system that is mediated by antigen-specific T and B cells and is observed only in vertebrates. In contrast, innate immunity is conserved from invertebrates to vertebrates. Even invertebrates and plants harboring only partial innate immunity have an effective host defence system. Studies of the host defence system in fruit flies (Drosophila) provided the first clue as to the mechanism of innate immune recognition.

Toll was initially identified as an essential protein that plays a central role in the establishment of dorsoventral polarity in the embryo of Drosophila. In Drosophila, a family of Toll receptors plays an important role in combating the invasion of pathogens (1). Adult fruit flies which are mutated in Toll are susceptible to infection by fungi and bacteria, respectively (2-3). It indicates the importace of Toll in protection of Drosophila against infection.Subsequently, homologues of Drosophila Toll were identified in mammals and are termed Toll-like receptors (TLRs) (4). TLRs compose a large family with at least 11 distinct TLRs (PAMPs) have now been identified in humans and 13 in mice.

Among the 11 known mammalian TLR family members, TLR2, 4, 5, 6 and 9 have been implicated in the recognition of bacterial components. TLR2 is responsible for the recognition of peptidoglycan and lipoprotein, whereas TLR4 recognizes LPS. TLR3 is implicated in the recognition of dsRNA and viruses which is produced by most viruses during their replication and TLR9 is a receptor for CpG DNA. TLR5 has been shown to be a receptor for flagellin in bacteria.

Several ligands have been characterized as TLR7 and/or TLR8 ligands, classified in synthetic compounds and natural nucleoside structures. Most or all of the TLRs, like Toll are believed to be functional multimers. Some, like the TLR2 complexes with TLRs 1 or 6, are heteromeric. Some appear to be homomeric, and in some cases, non-TLR subunits are part of the signaling complex. For example, TLR4 seems not to detect LPS directly, but only as a complex with MD2 and CD14, a small tightly associated LPS binding subunit. (2, 5-11).

Some synthetic compounds were already produced and used as immune activators before they were characterized as TLR7/TLR8 ligands. TLR10, which exists in humans and is most closely related to TLRs 1, 2, and 6, has been lost from the mouse genome. Its ligand cannot be explored in the mouse and remains uncertain. TLRs 11, 12, and 13 have been lost from the human genome, and of the 3, only one ligand for TLR11 has been identified (11-12).
They detect a broad range of pathogen-associated molecular patterns (PAMPs) to recognize different microbial as a means to distinguish 'non-self' from 'self., and in some cases they also recognize endogenous ligands, which are considered damage-

associated molecular patterns (DAMPs) (10, 13). PAMPs are integral structural components of the pathogens and are thus essential to the survival of the infectious organisms. Therefore, PAMPs are expected to be conserved among a range of pathogens, including virus, bacteria and fungus (10).

TLRs act as primary sensors of microbial products and activate signaling pathways that lead to the induction of immune and inflammatory genes (10). TLRs belong to a broader family of proteins, which include receptors for the pro-inflammatory cytokines IL-1, IL-18 and the orphan receptor T1/ST2 (14). All members of this superfamily signal inflammation in a very similar manner. This is due to the presence of a conserved protein sequence in the cytosolic domain called the Toll/IL1 receptor (TIR) domain, that activates common signaling pathways, most notably those that activate the transcription factor NFκB and stress-activated protein kinases (14).

It was initially thought that TLRs are primarily expressed by antigen-presenting cells (APCs), such as macrophages and dendritic cells, and that interactions between microbial ligands and TLRs in these cells will indirectly result in activation of cells of the adaptive immune system, especially T cells. Evidence is now accumulating that TLRs play an important role in the recognition and activation of components of pathogens not only in innate immunity but also in adaptive immunity. It has now become clear that TLRs are also expressed by various T cell subsets, such as conventional αβ T cells, regulatory T cells, CD8 T cells (15-17), and γδ T cells as well as natural killer T cells (18-19). Importantly, it appears that at least in some of these T cell subsets, TLRs are functionally active, because stimulation of these cells with TLR agonists in the absence of APCs results in exertion of effector or regulatory functions of T cells.

METHODS

Toll-like Receptors on T cells

Most investigations on TLRs have focused on cells of the innate system because TLRs are closely associated with innate response. However, there is no *a priori* reason why TLRs may not have a direct function in adaptive immunity. Expression of TLRs on innate and adaptive immune cells seems to be important in immune systems for elimination of pathogen. TLRs are expressed widely in many types of immune cells, including DCs, neutrophils, eosinophils, mast cells, macrophages, monocytes and epithelial cells (9-10, 20). Interestingly, we and others reported TLR express functionally on different subtype of T cells. TLR-3, -6, -7 and -9 have been reported to be expressed on CD4+ T cells (21).

TLR messages have been sporadically reported in T cells (15-16, 18, 22-25). We have for the first time demonstrated that TLR2, and TLR2 only**,** is functionally expressed on the surface of activated T cells and memory cells (16-22). Resting naïve human $CD4^+$ T cells (99.9% pure from human cord blood) express intracellular TLR messages but no detectable cell surface TLRs. However, following a few hours activation *in vitro* with plate-bound anti-CD3, and particularly in the presence of IFNα, these cells express clear cell surface TLR2 and TLR4 as shown by flow cytometry and immunofluorescence microscopy (15-17). Such cell surface expression was also

seen with Epstein-Barr virus (EBV)-specific human $CD8^+$ T cells following re-stimulation with EBV peptide *in vitro*. This finding suggests clinical relevance of TLR expression in T cells. Activated T cells responded to BLP (synthetic bacterial lipoprotein, $Pam_3Cys\text{-}SK_4$, a specific TLR2 ligand) to proliferate and produce markedly enhanced levels of cytokines, including IL-2, IFNγ, and TNFα. In contrast, activated T cells did not respond to LPS (a TLR4 ligand). This is most likely explained by the lack of CD14 (a co-receptor of TLR4) expression on T cells. The BLP-induced T cell proliferation can be specifically blocked by anti-TLR2 antibody, and is unlikely to be mediated by potential contamination of antigen presenting cells (APC), since anti-CD3 activated T cells from TLR2 knock out mice did not respond to BLP even in the presence of 5% APC from wild-type mice. We then went on to show that $CD4^+CD45RO^+$ memory T cells from adult peripheral blood constitutively expressed TLR2 and rapidly produced more IFNγ in response to BLP than naïve $CD4^+CD45RA^+$ T cells cultured with immobilised anti-CD3 antibody. Interestingly, BLP also significantly enhanced the proliferation and IFNγ production of memory $CD4^+CD45RO^+$ (but not naïve $CD4^+CD45RA^+$) T cells cultured with IL-2 or IL-15 alone, in a bystander manner.

These results, therefore, show that TLR2 serves as a co-stimulator receptor for antigen-driven T cell development, and may help maintain T cell memory. These finding suggests that pathogen, via their PAMPs, may contribute directly to the activation and perpetuation of T cell memory in antigen dependent and independent manner. It should also be noted that BLP alone did not activate naïve or memory T cells. It does so only in the presence of TCR activation or via a bystander effect of cytokines such as IL-2 or IL-15.

This dual-signalling mechanism should avoid excessive T cell proliferation by BLP alone (15-16, 22). We also have preliminary data showing that BLP could enhance the proliferation and survival of memory T cells *in vivo,* in an adoptive transfer of OVA/TCR transgenic (D0.11) mouse model (Komai-Koma M et al., unpublished data). By contrast, TCR stimulation down-modulates significantly surface TLR-5 expression on human CD4+ T cells (29). TLR expression on T cells may be regulated by TCR signalling, which needs further investigation in the future. These data thus offer the possibility that pathogens, via their PAMPs, may contribute directly to the perpetuation and activation of T cells.

At least some TLRs may function as a co-stimulatory receptor for antigen-specific T cell responses and participate in the maintenance of T cell memory (15, 30-31). It has been shown that ligands for TLR-2, -3, -4, -5 and -9 enhance the proliferation and/or biological functions of conventional effector T cells (15, 17, 30, 32). Co-stimulation of CD4+ T effector cells with anti-CD3 mAb and TLR-5 ligand flagellin results in enhanced proliferation and production of IL-2 at levels equivalent to those achieved by co-stimulation with CD28 (33-34).

CpG-containing oligodeoxynucleotides (CpG-ODN) can co-stimulate primary T cells in the absence of APCs (35). In the presence of the TCR signal, CpG-ODN induces IL-2 production, IL-2R expression and thus T cell proliferation. Furthermore, CpG-co-stimulated T cells differentiate into cytolytic T lymphocytes in vitro (35). Co-stimulation of antigen-activated murine CD8+ T cells

with the lipopeptide Pam3CysSK4 (Pam), a TLR-1/2 ligand, enhances the proliferation, survival and effector functions of these cells (17-33) TLR-2 engagement on CD8+ T cells reduces significantly their need for co-stimulatory signals delivered usually by mature APCs (17).

It was reported that activated neonatal naive $CD8^+$ T cells are functionally responsive to direct stimulation by TLR2 or TLR5 agonists. Flagellin and Pam_3Cys functioned directly to enhance cellular activation, clonal expansion, and cell effector function beyond that which was achieved by normal cellular activation. They suggest that the combined and sustained dual stimulation of this cell type may represent an attractive new avenue in adjuvant design for future neonatal vaccination strategies requiring a $CD8^+$ component (33). It is also reported that TLR3 agonists might also directly influence some $CD8^+$ T cell effector functions. The increased IFN-γ production provided by TLR3 signaling in $CD8^+$ effector T cells which could be beneficial in therapeutic vaccines, and may lead to better responses against tumors or chronic viral infections (32). Application of bacteria and their product such as LPS and Lipid A is not new concept in immunology and was practiced before. Traditionally, activation of TLRs in APCs would lead to the production of IFN-α, pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6, and the cytokines IL-12 and IL-18 that instruct Th1 to differentiate, whereas an increased Th2 response was observed in MyD88 deficient mice with impaired TLR signaling (36-37).

Moreover, it has been demonstrated that the dose of antigen plays an important role in directing Th1/Th2 differentiation driving by DCs. A lower concentration of ovalbumin (OVA) peptide (1 and 10 ng/mL) induce Th2 commitment while higher concentrations (1 μg/mL and 100 ng/mL) failed to elicit Th2 development. Stimulation of $CD4^+$ T cells with DCs along with TLR2 or TLR9 agonists in the presence of the 10 ng/mL of OVA peptide, the optimal antigen concentration for Th2 development resulted in suppression of IL-4 production and Th2 development. This suggests that TLR-activated DCs can block Th2 lineage commitment independent of antigen dosage (39). A lower dose of LPS (0.1 μg), through TLR4 signaling, induced a Th2 response to inhaled antigens in a murine allergic sensitization model.

In contrast, high doses of LPS (100 μg) with antigen resulted in a Th1 response (40). However, repeated administration of TLR2 ligand Pam_3CSK_4 or TLR4 ligand LPS leads to tolerance of TLR2 or TLR4 (41-42) with reduced cytokine release and expression of IRAK-1 and IRAK-4 proteins (41). Additionally, activation of TLR4 resulted in a MyD88-dependent Th17 response in memory $CD4^+$ T cells in the absence of TRIF molecule (38).

RESULTS

TLR and $CD4^+CD25^+$ Treg cells

There is considerable interest in the functional role of regulatory T (Treg) cells, which subsume the role of the much-maligned suppressor T cells. There are currently at least three major types of Treg cells: Th3, Tr1 and $CD4^+CD25^+$ T cells with overlapping functions (43-45). $CD4^+CD25^+$ T cells are arguably the best characterized so far and have been implicated in the prevention of a range of inflammation in infectious and autoimmune diseases (46). $CD4^+CD25^+$ Treg cells are found in mice and

human and represent 5-10% of peripheral blood $CD4^+$ T cells and are regarded as memory T cells. These Treg cells originate from the thymus through intermediate-affinity selection and are hypo-responsive to allogenic or polyclonal activation *in vitro*. However, they suppress the proliferation of conventional $CD25^-$ T cells in co-culture in a cell-contact dependent and antigen nonspecific manner.

The exact mechanism by which $CD4^+CD25^+$ Treg cells exert their suppressive effect is unclear but may involve the inhibition of IL-2 transcription in the responder cell populations. The suppressive function is critically dependent on the presence of Foxp3 (47-48). $Foxp3^{-/-}$ mice failed to produce $CD4^+CD25^+$ Treg cells and developed spontaneous autoimmune diseases. We reported that $CD4^+CD25^+$ Treg cell suppress the differentiation and function of Th1 and Th2 cells, *Leishmania major* infection and colitis in mice (49). We also have found that $CD4^+CD25^+$ Treg cells can be directly activated by BLP (but not LPS) and that this may be correlated with the expression of Foxp3.

Furthermore, we found that BLP, together with anti-CD3 antibody, could activate Treg cells but reversibly abolish the suppressive activity of these cells. This series of study demonstrate that TLR2 provides a strong positive signal for the amplification of T cells response (16, 50).

On the other hand, engagement of TLR2 resulted in human $CD8^+CD25^+Foxp3^+$ Treg cells expansion that directly suppressed $CD4^+$ T-cells proliferation by cell-contact inhibition and triggered $CD4^+CD45RO^+$ memory T-cell apoptosis inhibiting allergen induced Th2 immune responses (51). Treg cells are able to regain their suppressive property in the presence of IL-2 once the TLR2 ligand is removed (16, 52). Although TLR2-stimulated Treg cells readily lost their ability to suppress proliferation of effector T cells, cytokine production by effector T cells was still repressed.

This suggests that the activity of Treg cells was cytokines independent (53). Treg and Th17 cells are considered divergent and mutually inhibitory. It has been reported that when naive $CD4^+$ T cells were stimulated with TLR2 agonists, Th17 differentiation in vitro and Th17 cytokine production occurred (54). Thus, the reduced suppressive function of Treg cells induced by TLR2 stimulation may be a result of imbalanced phenotype and function between Treg and Th17 (55).

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Review Article

Role of Glucagon like peptide -1 (glp1) in health, disease and weight loss

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دور البيبتيد شبيه الجلوكاغون (GLP1) في الصحة والمرض وفقدان الوزن

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الملخص

يبدو نظام البيبتيد شبيه الجلوكاغون 1 (GLP1) معقدا للغاية. هذا النطام له تأثير إيجابي على توازن الطاقة الذي يؤدي إلى فقدان الوزن وتحسين التحكم الأيضي. المعرفة بهذا النظام يمكن استغلالها علاجيا لكل من البدناء والاشخاص المصابين بالداء السكري من النوع 2 .العقاقير المستعملة في علاج الداء السكري (ما عدا الميتفورمين ومثبطات4-DPP) ، ترتبط عادة مع زيادة الوزن.

منبهات مستقبلات البيبتيد شبيه الجلوكاغون 1 تساعد في تحسين السيطرة على نسبة السكر في الدم وترتبط مع فقدان الوزن .تدل الكثير من الأدلة والخبرة السريرية على ان منبهات مستقبلات لببتيد شبيه الجلوكاجون 1 (GLP1) يمكن أن تؤدي إلى تحسينات كبيرة في مستوى الهيموجلوبين السكري (HbA1c) فضلا عن الآثار المفيدة المستمرة على وزن الجسم.

القدرة على تحقيق فقدان الوزن في سياق تحسين السيطرة على مرض السكري هو سمة مهمة ومرغوب فيها من أي تدخل علاجي .هذا الاستعراض يساعد في إظهار آثار البيبتيد شبيه الجلوكاغون 1 (GLP1) وقابليته على فقدان الوزن المحتملة في ضوء الدراسات المختلفة.

ABSTRACT

Objective:
The endogenous GLP-1 system appears to be highly complex. It has a positive impact on energy homeostasis that leads to weight loss and improved metabolic control. This knowledge can be exploited therapeutically for both obese people and subjects with type 2 diabetes. Diabetes therapies, apart from metformin and DPP-4 inhibitors, are associated with weight gain. The GLP-1R agonists improve glycemic control and are associated with weight loss. Accumulating evidence and clinical experience for glucagon like peptide 1 (GLP1) receptor agonists shows that they can effect considerable improvements in glycated hemoglobin (HbA1c) levels as well as sustained beneficial effects on body weight.

The potential to achieve weight loss in the context of improved diabetes control is an important and desirable characteristic of any therapeutic intervention.

This review help in showing the effects of GLP 1 and its weight loss potential in the light of different studies.

Keywords: *Glp1, Exendin 4,Type2 Diabetes*

INTRODUCTION

Researchers theorized that the gastrointestinal tract might release a hormone in response to glucose that could stimulate insulin secretion above and beyond that stimulated by glucose alone. This then-undiscovered hormone was called "incretin." The incretin GLP-1(glucagon like peptide) was found to have a profound effect on stimulating the release of insulin from the pancreas. (1)

The glucagon-like peptide-1 (GLP-1) receptor agonists are a new class of injected drugs for the treatment of type 2 diabetes. They have additional effects in reducing glucagon, slowing gastric emptying and inducing satiety. In clinical practice they are associated with significant reductions in glycosylated haemoglobin (HbA 1c), weight loss and a low risk of hypoglycaemia. Beneficial effects have also been observed on blood pressure and lipids.

A solution to the issue of GLP-1's short action time came from an unusual source. Scientists working on toxins in the saliva of the lizard Heloderma suspectum, otherwise known as the Gila monster, found a protein that activated GLP-1 receptors. This protein, named exendin-4, originates in the salivary glands but has endocrine effects. (1)

Exendin-4 is a protein composed of 39 amino acids that mimics many of the actions of GLP-1 but that, unlike GLP-1, has a prolonged half-life in the bloodstream (meaning it remains in the blood for longer). Exendin-4 has several properties that mimic those of GLP-1. These include the stimulation of insulin secretion, the suppression of glucagon (a hormone that signals the liver to release glucose when blood glucose levels drop) secretion, and the slowing of stomach emptying.

PHYSIOLOGICAL ACTIONS OF GLP-1

Glp-1 Has Multiple Physiological Effects

1. It increases insulin secretion while inhibiting glucagon release.
2. Lower plasma glucose while reducing the likelihood of hypoglycemia.
3. It delays gastric emptying and food intake is decreased after GLP-1 administration .
4. It also Promotion of β-cell proliferation and reduced β-cell apoptosis .All patients were subjected to the following:

With respect to β-cell function, GLP-1 rapidly and potently stimulates insulin secretion. However, GLP-1 also stimulates insulin gene transcription, islet cell growth, and neogenesis, additional potentially important functions that may be clinically relevant for the treatment of diabetes. GLP-1 appears to improve insulin sensitivity and glucose uptake of both human and rat adipose tissue and skeletal muscle. (2) Several studies suggest that GLP-1 may directly enhance glucose disposal in an insulin-independent fashion, although this may also result from the overall inhibition of glucagon secretion. (2) Administration of GLP-1 agonists also leads to decreased hunger and increased satiety. (3)

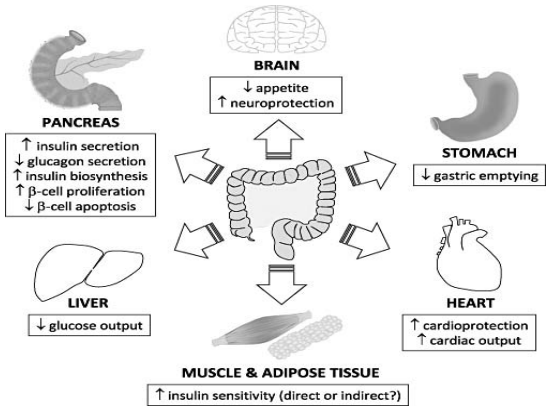

Figure source Emerging cardiovascular actions of the incretin hormone glucagon-like peptide-1: potential therapeutic benefits

EFFECT OF GLP1 IN TYPE 2 DIABETES.

It is well known that type 2 diabetes is characterized by defects in both insulin secretion and peripheral insulin sensitivity. Current treatments for the β-cell defect include the sulfonylurea (SU) drugs, which were the first therapy targeted against insufficient insulin secretion. However, these compounds promote insulin secretion independent of blood glucose and can, therefore, cause hypoglycemia.(4)

The properties of GLP-1, including glucose-dependent stimulation of insulin secretion and the expansion of β-cell mass, coupled with the inhibition of glucagon secretion and food intake, suggest that it would greatly complement current β-cell therapies. Trials with GLP-1 in diabetic patients have shown it to stimulate insulin secretion, inhibit gastric emptying, lower circulating glucagon, and improve overall glycemic control through both intravenous and subcutaneous injection.(5)

EFFEECT OF GLP 1 ON CVS

Although the major physiological function of GLP-1 appears to be in relation to glycaemia control, there is growing evidence to suggest that it may also play an important role in the cardiovascular system. GLP-1 receptors (GLP-1Rs) are expressed in the heart and vasculature of both rodents and humans. Recent studies have demonstrated that GLP-1R agonists have wide-ranging cardiovascular actions, such as modulation of heart rate, blood pressure, vascular tone and myocardial contractility. Importantly, it appears that these agents may also have beneficial effects in the setting of cardiovascular disease.(6)

EFFECT ON KIDNEY

GLP-1 can induce protective actions on the glomerular (renal) endothelial cells by inhibiting the signaling pathway of Ang II and its pro-inflammatory effect; and demonstrated a dual signaling mechanism by which hyperglycemia, via PKC β (protein kinase c) activation, can increase Ang II action and inhibit GLP-1's protective effects by reducing the expression of GLP-1 receptors in the glomerular endothelial cells.(7)

GLP 1 AND ITS MECHANISM OF WEIGHT LOSS

The principal action of GLP-1 agonists is mediated via their inhibition of eating. In searching for the underlying mechanism of GLP-1 receptor agonist induced anorexic effect, scientists have discovered pathways in the central nervous system, as well as in the periphery.(8)

The potent dose-dependent inhibition of gastric emptying observed following GLP-1 infusion in human subjects with Type 2 diabetes produce significant lowering of meal-related glycemic excursion, even without any increase in levels of circulating insulin.(9)

The inhibitory effects of GLP-1 on GI motility are also detected in human studies in the inter-digestive state. (10)

Administration of GLP-1 agonists also leads to decreased hunger and increased satiety in both the fed and fasted state; it appears that retarded gastric emptying is not the sole explanation of GLP-1–induced anorexia

Some studies suggest that GLP-1 has effects in appetite control centers in the human brain. Pannacciulli et al studied 42 healthy, normal volunteers using positron emission tomography imaging of the brain and demonstrated that peak postprandial increases in plasma GLP-1 concentrations were correlated with increases in regional cerebral blood flow in the left dorsolateral prefrontal cortex and the hypothalamus, areas previously shown to be related to food intake.

Effect on Hb A1C levels

Data from published clinical trials using long-acting GLP-1 receptor agonists (liraglutide, exenatide, albiglutide, taspoglutide, reveal that reductions in A1C from baseline range from −0.87 to −1.9%.

Results with exenatide demonstrated that these improvements in A1C could be maintained after 2 years (mean A1C decrease at 2 years: −1.8%) (11).

Greater reductions in A1C were seen with liraglutide compared with the DPP-4 inhibitor sitagliptin (mean A1C decrease: −1.50 and −1.24% with 1.8 and 1.2 mg liraglutide, respectively, vs. −0.90% with sitagliptin) (12).

Summary

Endogenous GLP-1 released from entero endocrine cells is a prandial satiety hormone

1- In the periphery, satiety including effects of GLP-1 are likely to be mediated via vagal apparent originating in the intestine

2- In the central nervous system, ascending GLP-1 containing pathway arising in the dorsal vagal complex is a mediator of satiety

3- Both central and peripheral GLP-1 receptors are valid targets for weight management therapies

GLP-1 MIMETICS AND WEIGHT LOSS IN CLINICAL TRIALS

Most clinical trials of GLP-1mimetics have demonstrated modest weight loss.
With exenatide, the phase III studies indicate that weight loss occurs particularly when exenatide is given as mono therapy or combined with metformin therapy.(13)

GLP-1 is secreted into circulation after food intake. In the pancreas, GLP-1 stimulates glucose-induced insulin secretion (an incretin hormone) and inhibits glucagon secretion, thereby substantially contributing to maintaining the glucose homeostasis (14).
Activation of both peripheral GLP-1 receptors and GLP-1 receptors in the central nervous system reduces appetite and food intake thereby ensuring that body weight is kept down. There are 2 principal mechanisms by which GLP-1 can have weight-related effects. GLP-1 has been shown to have important effects on the GI system as well as the central nervous system.(14) The infusion of GLP-1 has been shown to decrease the rate of gastric emptying and to reduce acid secretion.
This, in turn, is expected to lead to an increase in satiety and, thus, decreased food intake (15)
Clinically, the degree of weight loss appears to be positively correlated with the dose of GLP-1 analog. A recent two-year prospective study (including non-diabetic patients with baseline BMI ≥30) randomized to treatment with the GLP-1 analog liraglutide (2.4 to 3.0 mg once-daily) reported a weight loss of 7.8 kg (compared to a 2 kg weight loss in the placebo group) (16).

At present, six different GLP-1 analogs have been subject to trials: exenatide (Eli Lilly), liraglutide (Novo Nordisk), albiglutide (GlaxoSmithKline), taspoglutide (Ipsen and Roche), lixisenatide (Sanofi-Aventis) and LY2189265 (Eli Lilly). Only exenatide and liraglutide have been approved by the U.S. Food and the Drug Administration and European Medicines Agency.

GLP-1 analogues have the indication type 2 diabetes in combination with metformin and/or sulphonylurea, when treatment with these drugs is insufficient. Much academic and commercial effort is being put into investigating the possibility of extending the indication to obesity.

EXANATIDE

Both exenatide and liraglutide are administered subcutaneously; exenatide was shown to reduce weight by approximately 1.5-3.0 kg over a 30-week period. Exenatide exists in two formulations: twice daily (Byetta®) and once weekly (Bydureon®).

LIRAGUTIDE

Liraglutide, a glucagon-like peptide (GLP-1) analogue, is a member of new classes of anti-diabetic agents and is characterized by induction of insulin secretion only during hyperglycaemia as an incretin effect. Liraglutide (Victoza®, Novo Nordisk A/S, Bagsvaerd, Denmark) is administered once daily. The Liraglutide Effect and Action in Diabetes (LEAD) studies have demonstrated a significant weight reduction by liraglutide (17). The LEAD (Liraglutide Effect and Action in Diabetes) program represents a large series of studies undertaken in approximately 4200 patients with type 2 diabetes to characterize the effect of liraglutide over the spectrum of type 2 diabetes care.

In a pilot study, it was recently reported that short-term liraglutide treatment reduced BMI, waist circumference, and visceral fat area, and reduced the scale for eating behaviour (18). However, this short-term study was performed only during hospitalization and thus it remains uncertain whether these effects of liraglutide are maintained after discharge. (19)

Potential clinical implications of GLP-1 analog treatment

First, encouraging and growing evidence supports that a sizable and enduring weight loss can be obtained by GLP-1 analog treatment in both diabetic and non-diabetic overweight or obese patients.

The results of the seven incretin-based clinical trials have generally demonstrated the same benefits observed in comparative trials with other glucose-lowering agents. More specifically, depending on background glucose-lowering therapy, a weight loss of 1–4 kg is observed in patients treated with a GLP-1 receptor agonist (20)

The major side effects of exenatide and liraglutide are mild to moderate nausea and vomiting. These side effects are dose-dependent and decline over time, and they do not explain the observed weight loss

Study design	Study results of weight loss	Lipid levels
Exenatide Versus Liraglutide (LEAD-6) Buse et al[22]	In the 26-week trial , weight losses of 2.9 and 3.2 kg were observed with exenatide and liraglutide, respectively. In the 14-week extension phase, those switched from exenatide to liraglutide experienced an additional weight loss of 0.9 kg compared to 0.4 kg for those who remained on liraglutide	Compared to exenatide, triglycerides were reduced significantly more with liraglutide, 1.8 mg daily
Liraglutide Versus Sitagliptin Pratley et al[23]	In the comparison of liraglutide with sitagliptin a weight loss of 2.9 kg was observed in the group taking liraglutide, 1.2 mg; a loss of 3.4 kg in the group taking liraglutide, 1.8 mg	
Exenatide Versus Sitagliptin[24]	In the crossover comparison of exenatide with sitagliptin, patients treated with exenatide for 2 weeks before the crossover lost 0.8 kg, and those treated with sitagliptin lost 0.3 kg	Compared to sitagliptin, exenatide resulted in a significantly greater reduction in triglycerides, whereas liraglutide resulted in a similar reduction after 26 weeks
The SCALE study (Satiety and Clinical Adiposity – Liraglutide Evidence in Non-Diabetic and Diabetic Subjects [25]	This study Investigated liraglutide's efficacy for weight loss in a particularly obese population. Baseline BMI of the SCALE patients averaged 38 kg/m2, while approximately one third of SCALE patients had a BMI of over 40 kg/m2.The result of a 5% weight loss was achieved	

associated with GLP-1 analog treatment (21).

The following table shows the results of some studies carried out to see the effect of Exanatide and Liraglutide on weight reduction.

A random meta-analysis including 3395 participants randomly assigned to GLP-1R agonists and 3016 assigned to the control groups, from 21 trials showed that the weighted mean change in body weight was larger for patients in the GLP-1R agonist group than for those in the control group.(26)

In summary, treatment with GLP-1 mimetic is associated with decreases in appetite and body weight. On average, the weight loss is modest, but in some individuals it can be significant. The effect appears to be dose dependent, with higher doses of GLP-1 mimetics associated with more weight loss.(27)

CONCLUSION

The endogenous GLP-1 system appears to be highly complex. GLP-1 has a positive impact on energy homeostasis that leads to weight loss and improved metabolic control. This knowledge can be exploited therapeutically for both obese people and subjects with type 2 diabetes.

Diabetes therapies, apart from metformin and DPP-4 inhibitors, are generally associated with weight gain. The GLP-1R agonists improve glycemic control and are associated with weight loss.

Accumulating evidence and clinical experience for glucagon like peptide 1 (GLP1) receptor agonists shows that they can effect considerable improvements in glycated hemoglobin (HbA1c) levels as well as sustained beneficial effects on body weight.

The potential to achieve weight loss in the context of improved diabetes control is an important and desirable characteristic of any therapeutic intervention. Further studies examining the impact of longer-acting preparations on long-term body weight will be of great interest.

In summary, treatment with GLP-1 mimetic is associated with decreases in appetite and body weight. On average, the weight loss is modest, but in some individuals it can be significant. The effect appears to be dose dependent, with higher doses of GLP-1 mimetics associated with more weight loss.(28)

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A case report

Primary Hepatic Carcinoid tumor: Dynamic CT and MRI findings

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الورم الكارسنويدي الاولي في الكبد: تقرير حالة

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الملخص العربي

الورم الكارسنويدي الاولي في الكبد يعتبر من الاورام النادرة جدا وعدد قليل منها قد تم توثيقة ونشرة في المجالات العلمية بعدد لا يتجاوز 60 حالة فقط.
نوجز هنا حالة مسجلة لورم كارسنويدي اولي في الكبد لمريض يبلغ من العمر 42 سنة قدم بأعراض الألم في البطن والقى والاسهال. تم تشخيص حالتة بأنة ورم سرطاني كرسنويدي أولي في الكبد علي اساس التحاليل الاشعاعية والمختبرية. تم إكتشاف ورم كبير في الفص الايسر من الكبد (الجزء 2 و3) ومستويات مرتفعة بشكل ملحوظ من (5-HIAA) في البول. أثبتت تحاليل الانسجة بعد الاستئصال الورم السرطاني الكارسنويدي الاولي في الكبد.

ABSTRACT

A 42-year-old man presented with abdominal pain, vomiting, and diarrhea. He was diagnosed with primary hepatic carcinoid tumor based on radiologic, laboratory and histopathological findings. Investigations showed a large mass in the left lobe of the liver (segment 2 & 3) and markedly elevated levels of 5-hydroxyindole acetic acid (5-HIAA) in the urine. Histologic and immunohistochemical findings of the resected left liver lobe mass showed a malignant carcinoid tumor. This case is of interest because of the rarity of this neoplasm. This case report describes and reviews the radiologic dynamic Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) features of this rare neoplasm.

Keywords: *Primary hepatic carcinoid tumor (PHCT), Focal Nodular Hyperplasia (FNH), Dynamic computed tomography, Dynamic Magnetic Resonance.*

INTRODUCTION

Carcinoid tumors develop from neuroendocrine cells and occur most frequently in the gastrointestinal tract in about 85% of cases, the respiratory system in 10% of cases and the rest inother various organs [1]. The vast majority of carcinoid liver lesions are metastatic in nature, but primary hepatic carcinoid tumors are extremely rare. Only few cases were reported since its first description by Edmondson in 1958 [2].

METHODS

A 42-year-old man presented to the surgery outpatient clinic with history of vomiting and diarrhea. The history also revealed abdominal pain over the last 6 month which was becoming more severe in the last two months before the presentation.

Physical examination demonstrated possible abdominal midline mass; otherwise the physical examination was normal with no clinical features of cirrhosis. Blood testing demonstrated mild anemia, however, the results of other laboratory investigations, including liver function tests and blood levels of tumor markers including α-fetoprotein, Ca-19-9, and carcinoembryonic anti-gen (CEA) were within normal limits.

Urinary excretion of the 5-HIAA over 24 hours sample collection (70mg/24h) was markedly elevated. The patient was referred to the radiology department for further evaluation of the midline abdominal mass by CT examination. The Non-contrast CT examination demonstrated a well encapsulated large left hepatic lobe lesion, involving segment 2 and 3 of the liver and measuring 12x13cm with central areas of low attenuations. (Fig.1A).

Subsequently, the patient went for a dynamic CT scan of the liver as per standard protocol. Arterial, Porto-venous and delayed images were obtained, and multiplanar reconstruction was performed.

On the arterial phase, avid enhancement of the lesion was seen with central frond-like projections were seen of low density (Fig. 1B). Direct supply of the lesion was seen by the left hepatic artery which was engorged and larger than the right hepatic artery (Fig 2A & B). On the porto-venous phase of the liver, the lesion showed no significant washout, and the central areas of low attenuations were found more conceptious and of lower attenuation relative to the rest of the lesion (Fig. 1C). Furthermore, the lesion showed well seen porto-venous supply, which was driven via the left portal vein, that wraps around the lesion and sends its branches into the lesion (Fig. 2C & D). On the delayed images, the lesion becomes homogeneous to the liver parenchyma with no change in the central frond like projections of low attenuation. (Fig. 1D).

At this stage and so far, the lesion was not behaving of a classical benign lesion and the central area of low attenuation further raised the suspicion as necrosis would be the main concern. This particular feature was more suggestive of either a primary malignant liver lesion or a metastatic lesion. After discussion with the referring surgeon, further evaluation by MRI was carried out especially, given the fact that Fibro Nodular Hyperplasia (FNH) was still in the differential diagnosis, although uncommon for the patient's gender.

On T1 weighted images, the lesion was encapsulated and shows inhomogeneous low signal intensity with lower signal intensity is seen centrally (Fig. 3A). On T2 weighted images, the entire lesion was of high signal intensity but centrally the lesion was of higher signal relative to the rest of the lesion (Fig. 3B). On the dynamic MRI post Gadolinium administration, the central area remained of low signal intensity throughout theglucagon like peptide dynamic imaging

with more homogeneous enhancement seen at the periphery of the lesion with a capsular enhancement seen persistently in all phases of the dynamic study with similar enhancement pattern to the dynamic CT examination seen on the arterial and the porto-venous phases (Fig. 3 C & D). The CT and MRI showed no radiologic features of liver cirrhosis. The patient refused the biopsy to obtain a tissue diagnosis as he was worried about the spread of the tumor. Given the fact that the CT and the MRI results were not reassuring and given the fact that the patient 5-HIAA was markedly elevated, the patient was booked for left hepatectomy. Meanwhile, more thorough investigations to rule out the possibility of metastatic carcinoid were performed; including upper and lower gastrointestinal endoscopies, CT Enteroclysis, CT chest, and Octreotide scintigraphy. They were all negative except for a focal uptake seen in the liver lesion on the Octreotide uptake scan. Surgery was performed, and the resected left lobe contained a solid tumor measuring 12x13cm (Fig. 4A, B, C & D). The microscopic and immunohistochemical findings were consistent with malignant carcinoid tumor of the liver. The final diagnosis in this case was primary hepatic carcinoid tumor. Annual follow-up of the patient over the last six years showed no local recurrence or metastatic disease.

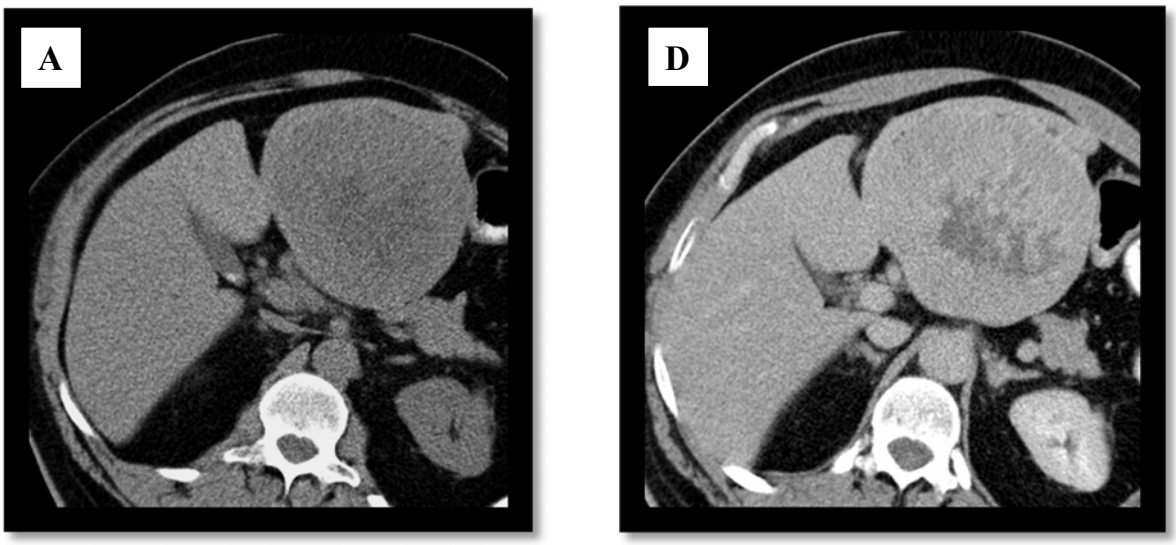

Figure 1. Dynamic Computed Tomography of the liver

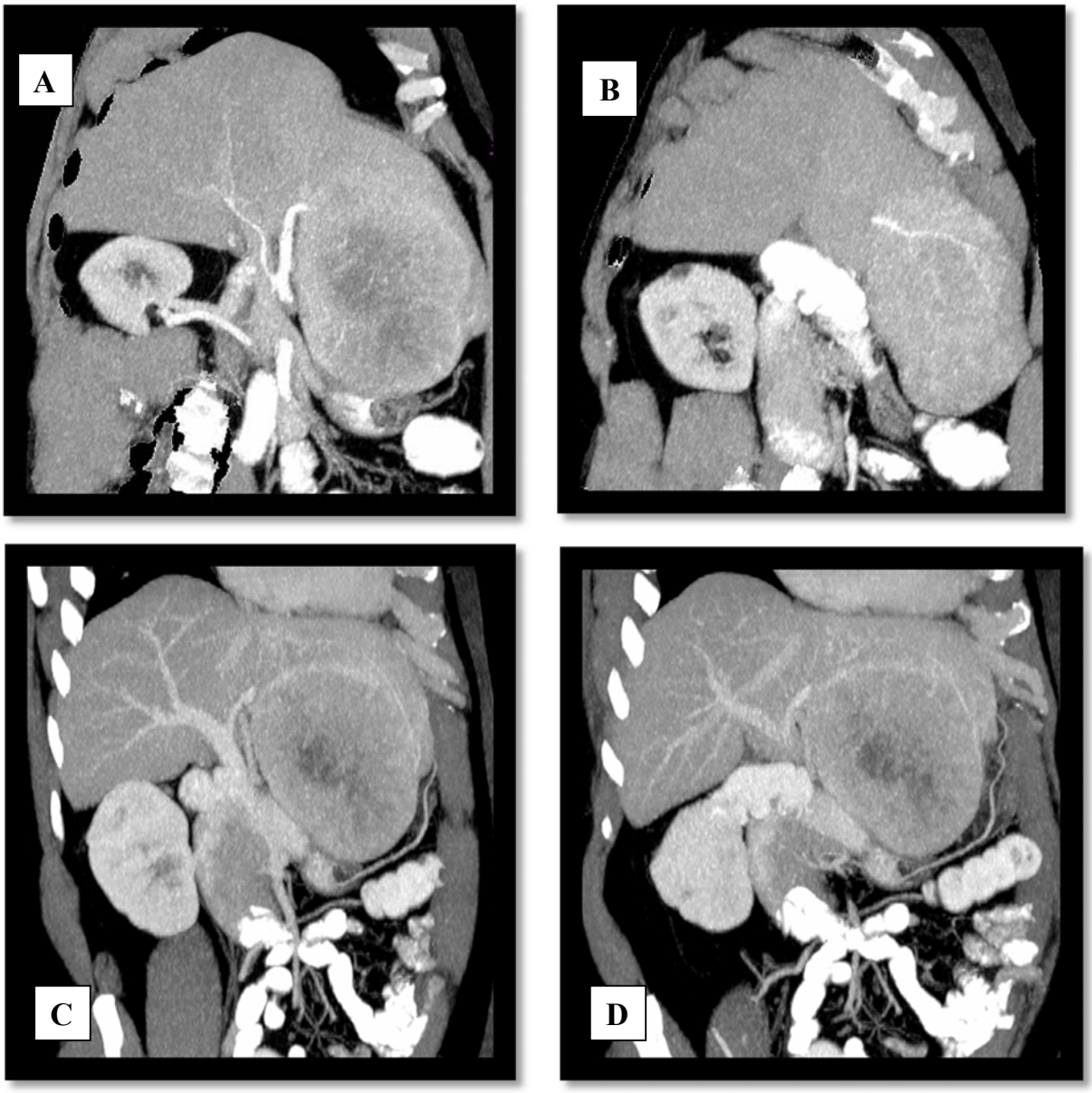

Figure 2. Reconstructed Dynamic Computed Tomography of the liver

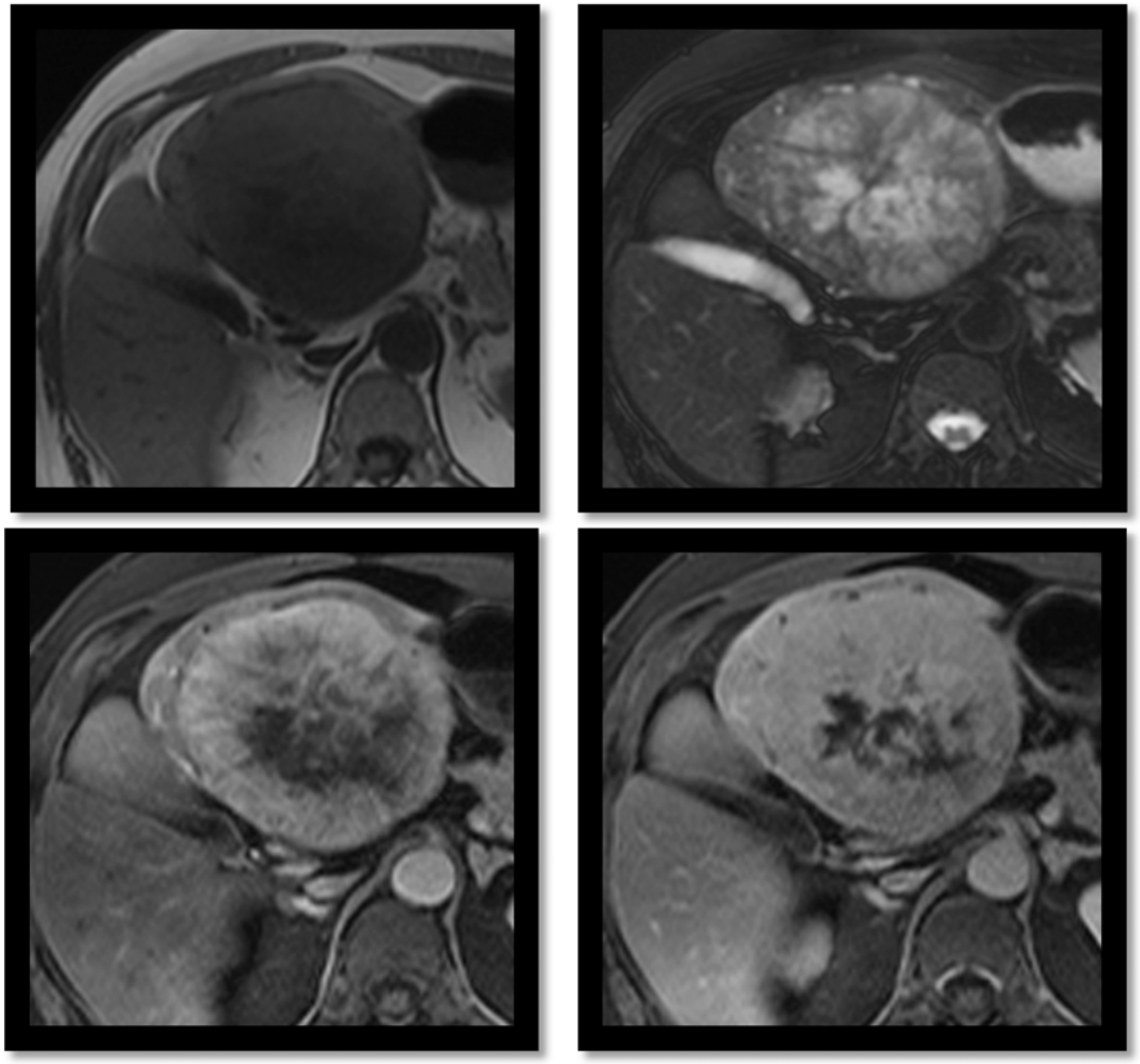

Figure 3. Dynamic Magnetic Resonance of the liver

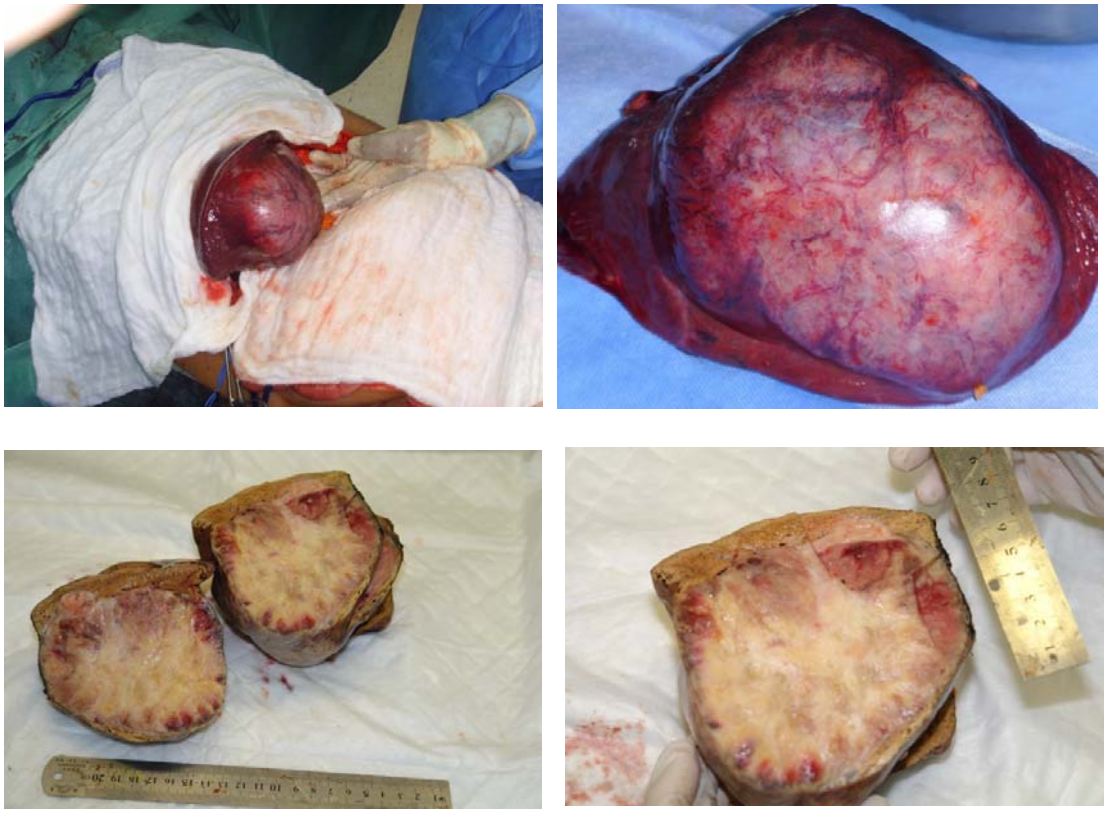

Figure 4. Gross specimen of the PHCT

DISCUSSION

Carcinoid tumors are rare slowly growing neuroendocrine neoplasms. Liver is the most common site for neuroendocrine tumor metastases, but primary hepatic carcinoid tumors are extremely rare [3]. There are about 60 cases reported in the literature [14]. On the previous reports, the CT and MRI findings concur with the findings of this report, but the features of these lesions are atypical and still can be confused with other malignant lesions such as Hepatocellular Carcinoma or benign lesions such as Focal Nodular Hyperplasia [4, 5, 6, 7 8, 9]. The main benign differential diagnosis in the present case was fibro nodular hyperplasia, which is a benign vascular hepatic neoplasm that is most prevalent in young women. The lesions consist of hepatocytes, bile ducts, blood vessels, and Kupffer cells and are characterized by scar tissue in the center [10]. Central area of low attenuation is seen in almost all of the previously reported primary liver carcinoid tumors, but their etiology differs as they were found to be related to necrosis within the lesion or related to central scaring [11]. In this reported case, the central area of low attenuation was the result of necrosis within the lesion, and this was clearly demonstrated on the MRI and pathologically correlated. The presence of a capsule was described and also identified in this case report. [4]. The majority of patients on the earlier reports were hormonally inactive [5], but in this report, the patient suffered from features of carcinoid syndrome, which were further

confirmed by the laboratory results that normalized after resection of the tumor.

Hormonally active tumor releases vasoactive substances into the systemic circulation. Serotonin (5-Hydroxtryptamine) is considered the main vasoactive substance responsible for the majority of symptoms encountered in patients with carcinoid syndrome; such as diarrhea, wheezing and those related to fibrotic reactions in the heart [12, 13]. Elevated metabolic breakdown of this active substance in the urine, namely the 5-Hydroxyindoleacetic acid over 24h is considered a highly reliable method for diagnosis [12, 13]. We noted that the majority of the previously reported primary hepatic carcinoid tumors were found in the right lobe of the liver, contrary to this case in which the lesion was in the left lobe of the liver.

CONCLUSION

Although common findings were observed in the literature for the reported cases of primary hepatic carcinoid tumors and agree with this report, the features are not specific and can be seen in benign and malignant lesions of the liver. Therefore, atypical lesions should be further evaluated by biopsy, and thorough investigations are strongly recommended if the carcinoid tumor is revealed. This is particularly true given the fact that the majority of carcinoid lesions in the liver are related to metastatic deposits. Treating the liver lesion without addressing the primary will have dire consequences.

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