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Mohamed Selim
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt., mohamed.star007@yahoo.com

Adel Mousa
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt., adel.abdelbaset@hotmail.com

Mohamed Mansour
Virology and Immunology Department, National Cancer Institute, Cairo University and Children's Cancer Hospital– 57357 Egypt.

Mohamed Farag
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.

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ADVANCED TRENDS FOR PRODUCTION OF HEPATITIS B VIRUS SUBVIRAL PARTICLES USING DIFFERENT TECHNIQUE TO ENHANCE OVERCOME OF HEPATITIS B VIRUS INFECTION

Mohamed A. Selim[1], Adel A. Mousa[1], Mohamed T. M. Mansour[2], Mohamed M.S. Farag[1*].

1. *Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.*
2. *Virology and Immunology Department, National Cancer Institute, Cairo University and Children's Cancer Hospital– 57357 Egypt.*

***Corresponding Author:** mohamed.farag@azhar.edu.eg.

ABSTRACT

Hepatitis B infection (HBV) disease is one of the main hazard factors for chronic hepatitis, liver fibrosis, cirrhosis and hepatocellular malignant growth (HCC), which is a significant worldwide medical issue Hepatitis B virus (HBV) infection is one of the leading risk factors for chronic hepatitis, liver fibrosis, cirrhosis and hepatocellular cancer (HCC), which is a major global health problemeven with vaccine use and self-resolution in most cases. HBV contains different particle forms including non-infectious spherical and tubular sub viral particles (SVPs) with 22-nm-diameter that present potent immunogenicity. The main goal of the presented study was to optimize the best route for creation the SVP which discharged from incorporated HepG2.2.15 cell line in lab. For aim achievement, incorporated HepG2.2.15 cell line refined for SVP creation and fixation. SVP analyzed by using serological marker and electron microscopy. Outcomes demonstrated the development of HepG2.2.15 cell line in our complete media (Williams E media) with heat inactivated fetal cow-like serum (FBS), insulin and hydrocortisone bring about creation high measures of discharged viral particles in the supernatant. For recognizable proof of morphology and structures of SVP, electron microscopy indicated various shapes including circular and fibers for having a similar morphology of HBV virion, while it's completely different in the diameter. These findings shed light on an important technique used in production of huge number of SVP, which is important advance in Hepatitis BV irus irresistible rounds, and the gathering of HBV subviral particles might be basically connected to pathogenesis of the virus inorder to enhance overcome of HBV infection.

Keywords: Hepatitis B Virus, Subviral Particles, HepG2.2.15cellline,Heat inactivation fetal bovine serum

1. INTRODUCTION

Around two billion individuals have been affected by the hepatitis B infection (HBV), even with antibody use and self-cured much of the time, more than 290 million individuals have ceaseless HBV disease [1] and are at higher danger of creating cirrhosis and hepatocellular carcinoma. Leeway of the hepatitis B surface antigen (HBsAg) from the blood is the standard for end of treatment in these patients[2]**.**furthermore, is the aim for new medicines to achieve absolutely cure of HBV infection [[3**.**HBV is an enveloped virus with an icosahedral core that enclosed on organized genome which contains 4 covering open understanding casings (ORFs) named S, C, P and X, separately[3].

Three types of viral particles are present in the blood of persons infected with HBV. The whole virion is the classical Dane particle, which is spherical in shape with a diameter of 42 nm. Virions have two layersan outer envelope, coated with hepatitis B surface antigen (HBsAg) proteinsand an inner nucleocapsid, composed of dimers of hepatitis B core antigen (HBcAg).HBcAg exists as two distinct populations that exhibit T3 or T4 symmetry, consisting of 180 or 240 core proteins, resulting in sizes of 32 nm or 36 nm, respectively[4]. . The nucleocapsid encloses the

HBV genome and intimately associated, endogenous DNA polymerase[4].Two subviral particles are also present in the sera, both of which are composed solely of HBsAg and are not infectious. One is a smaller spherical structure of 17–25 nm diameter, and the other is filamentous, being approximately 20 nm in diameter and of variable length. The function of these subviral particles is not clear but is probably immune decoys[4].The extreme creation of SVPs can cause immuneand, on the country, may be one of reasons of persistence of the chronic infection[5].The creation of the HBV surface proteins likewise prompts the intracellular maturing of void Subviral round or filamentous envelope particles (SVPs). SVPs are 20 nm in distance across, come up short on the nucleocapsid and are discharged in incredible overabundance over virions[6].

Resolving of HBV infections is required for cell-based immunity[7]. Subviral particles (SVP) are immunogenic and used as HBV vaccine [8]. The excess of SVP detected in patients and its biological function is unexplained and not understood at present. SVP might bind to the host neutralizing antibodies and increase the ability of the Dane particles to reach liver cells [9]. SVP might contribute to a state of immune tolerance that led to highly productive chronic infection[10]. Previous study with duck HBV reported that SVP could increase infection when found at low multiplicity. They were found to be inhibitory at their highest quantity[11].

In this study, we try to use HepG2.2.15 cell line to optimize the best route for production of Subviral Particles with different technique and characterize it using serological technique and Electron microscope to confirm the difference between Dan Particles in chronic HBV Patient and SVP produced from our cell line HepG2.2.15 which cloned with HBV DNA. Thus, assist to create immune therapy or and HBV vaccine depending On SVP and human blood samples to overcome the terrible impacts of Hepatitis BVirus.

2. MATERIAL AND METHODS:

2.1. Thawing and Propagation of cell line (HepG2.2.15)

HepG2.2.15culture obtained from a culture collection, was arrived frozen, the cryovial containing frozen HepG 2.2.15 cellsexpelled from liquid nitrogen and quickly set it into a 37°C water bath (< 1 minute). Then, vial transferred into a laminar flow hood; the outside of the vial wiped with 70% ethanol before opening. The thawed cells Pipetted dropwise into the falcon tube (15 ml capacity). Then slowly 5ml of 37°C pre-warmed Complete Williams E Medium (with 10%FBS heat inactivated at 56ºc for 30 minutes) was added. The cell suspension centrifuged at 1500rpm for 5 minutes. After the centrifugation, the supernatant discarded, the cells pellet resuspended in 7ml fresh growth medium, and then transfer to a 25 cm^2 cell culture flask, brooded overnight at temperature 37°C incubator with wet atmosphereCO2 (5%). After one-night incubation of HepG 2.2.15 adherent cells, 80% confluency degree assessed using an inverted phase contrast microscope and the absence of bacterial and fungal contaminants also confirmed. The spent cell culture media discarded from the culture vessel. Cell monolayer washed twice with 5ml PBS. The wash solution discarded from the culture flask. the cells were harvested by pipetting of trypsin/EDTA onto the washed cell monolayer, the flasks rocked 4–5 times to cover the monolayer. then the flask placed in a CO_2 incubator at 37°C for 2-10 min. under the inverted microscope, (≥ 90%) of the cells were detached and floated. Then, the cells resuspended in complete williams E medium (with 10% in activated FBS) to inactivate the trypsin. the cells transferred to a 15-mL falcon tube and centrifuged then at 1500rpm for 5 minutes. After the centrifugation, the supernatant discarded, the cells pellet resuspended in 1ml fresh growth medium for counting. The total number of cells per milliliter and percent of viability was determined using a hemocytometer, and trypan blue vital stain. the number of viable (seen as bright cells) were counted. After the calculations, the concentration of the vial cells was (6×10^6) cells/ml [12].

2.2. Optimizing the Production of SVP

These cells persistently produce and express HBVsvp into the supernatant finally. The HepG2.2.15was cultivated into three groups (75 cm2)(labelled Group 1, Group2, and Group3). Each group contains seven flasks (25cm2), each of which represents a specific day starting from day 1 to day 7. After that, 7ml of pre-warmed complete Williams E medium including heat in activated FBS were added in each flask at the level of three groups for different days. (0.750×106) cell/flask were added as initial seeding density of cell suspension for each one of the three groups, and the cells were incubated for up to 7days for all groups. Subsequently, the highest production rate of HBVsvp was determined for each day separately, finally, supernatant was stored at -80°C for later investigations, labeled with before concentration, in preparation to downstream HBVsvp quantification steps.

2.3. Growth curve assay

To determine the best conditions for production of SVB specific technique called growth curve assay was used[13].It was described as the following: Firstly, HepG 2.2.15 adherent cell line cultured in 2×75 cm2 culture flasks containing complete Williams E Medium (with 10% de activated FBS) and incubated for three days. Once cells became 80%- 90% confluent (the log phase), cellsre-suspended in 1ml complete medium, and the total number of cells and percent viability were counted using a hemocytometer, where 99ul cell suspension stained with 1ul of Trypan blue. (17×106/flak) total 34 per 2 flasks. Then the cells were plated at a density of variety number of cells per well (1470 to 752.640) cells distributed in 2×96 well plate to decide the expansion parameters by the MTT strategy.

2.4. The MTT assay

This was carried according to protocol Guide: MTT assay for cell viability and proliferation, at the beginning 200µl complete Williams media added per each well. Then The cells were plated at a density of variety number of (1470 to 752.640) cells per well. In duplicate, 200µL of the dilutions (1:10) Placed into entire wells (96×2) of a microtiter plate in addition to three control wells of 200µl of dH2O Included to provide the blanks for absorbance readings. the cells among 2 microtiter plates were incubated for 24 hours (to recover and reattach).The tests were done including 10 µL of MTT [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] (acquired from SIGMA-Aldrich) arrangement (5 mg/ml) for each well, at that point hatching for 4 hours. Framed formazan precious stones were broken down with 100 µL of isopropyl liquor at 0.05 N HCl, and the absorbance measures were enlisted at 570 nm on a miniaturized scale plate peruser (Thermo Scientific Multiskan Spectrum) utilizing the frequency of 570 and 590 nm as reference (Mossman, 1983), the information results were prepared on a Skan It Software 2.4.2. To decide the counter proliferative movement for the various concentrates, the phone lines were seeded (per triplicates) at a thickness of 10 × 104 cells/well (50 µL) in 96 well plates, and permitted to follow during 24 h at 37°C with 5% of CO2 and 95% of relative moistness. After 24 h of pre-brooding, 50 µL of the example separates were added at particular fixations to the cell-lines, and the time of hatching proceeded for another 48 h. All examines were completed in the "log" cell developing stage. The rate restraint of multiplication was determined looking at the absorbance estimations of the control (cell line without test extricates) and the test samples[12].

2.5. HBVsvp Concentration

HBVsvp were set up for concentration by the polyethylene glycol convention (PEG), the gathered medium hatched medium-term at 4°C with (40%) PEG (Nice, India). On Second day, supernatant centrifuged at 8000 rpm for 1 hour at 4°C. After centrifugation, the pellet was resuspended in 1x PBS (75%) containing in heat in activated FBS (25%), trailed by brooding for a night at 4°C. On third day, the segregates were gathered, and exposure again to centrifugation (4000 rpm for 20minutes) at 4°C. Finally, the detaches were gathered and put away at (- 80°) for serological measurement later[14].

2.6. Serological detection of HBsAg

All samples were assayed for (HBsAg)positivity by ELISA (Cam Tech Medical). It was carried according to protocol [15]. Briefly, all reagents and specimens equipped at room temperature before beginning the assay. The incubator adjusts at +37°C for incubation stage.Two well for positive and negative controls and one well for each specimen. The loading plate stage began with the addition of 50ul of the Negative Control,50ul of the Positive Control and 50ul of21specimens to respective wells. Then, 50ul of Anti-HBs Ag peroxidase-conjugate solution pipetted and mixed into each well used. Plate subsequently taped, labeled and incubated at 37° for thirty minutes. At the end of the incubation, the strips were manually washed 6 times with the working washing solution. Afterwards,50ul of Substrate solution A and B distributed on each well and incubated for10 min. at 37°C. After incubation, the response was halted by adding50ul of Blocking Reagent to each well. finally, the color developed on the microplate reader readied at 450 nm. The reading was doneon ELISA reader within 30 minutes from the stop reaction was added[15].

2.7. Morphological characterization of HBV

Various examples from constant HBV patients (one test)and SVP delivered cell line(one test)/,analyzed and portrayed by TEM (JEOL 1010, Nano innovation focus/Egypt) at 200 Kilo Volt to Differentiate between size and shape of particles for both Chronic samples and HBSVP (with two each types spheres and filamentous)samples. The examples were set up by drop the examples supernatant onto the carbon-covered copper grid, stacked onto an example holder and scanned by TEM [16].

3. RESULTS:

3.1. Serological detection of HBsAg:

All samples were assayed for (HBsAg)positivity by ELISA following the manufacturer's instructions.The reading was done within 30 minutes from adding the stop solution. The highest viral production was 1.365 at day seven, and there we discussed the average of all groups (three group) at table one.

3.2. Sub Virus particles (SVP) Production and Optimization:

To configure the optimum conditions for determination of the highest productivity of HBSVP over 7 days cells culturing. The highest production rate of HBSVP was determined for each day separately as a following: Cells of day one from the 3 groups were examined under inverted microscope for assessing cells proliferation rate. And then photographed using 10X, 20X, and 40X magnification. Then, 7ml of cell culture supernatant (containing HBSVP) harvested from day 1 flask of the three groups. then, the same previous steps were repeated for the rest of the days (from day 2 to day 7) among the three groups separately, and finally, 7 falcon tubes (containing 6ml cell culture supernatant)and 7 Eppendorf tubes

Table (1): the average of HBsAg quantification (optical density).

HBsAg quantification (OD)								
Groups	**Cells no.**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	D7
1st Group	**$0.75X10^6$**	0.034	0.109	0.445	0.595	0.784	0.914	1.32
2nd Group	**$0.75X10^6$**	0.047	0.129	0.246	0.518	0.765	1.203	1.365
3rd Group	**$0.75X10^6$**	0.003	0.038	0.078	0.226	0.821	1.118	1.097
Average		**0.028**	**0.092**	**0.306**	**0.5103**	**0.79**	**1.0783**	1.260

labeled with before concentration for assessment of HBSVP (1.5ml) (containing 1ml cell culture supernatant)in triplicate stored at -80°C in preparation to downstream HBSVP Quantification steps. Finally, the best day for production was day 7.

Photograph (2) Showed Morphology of prolonged HepG2.2.15 cells measured by stage differentiate light altered magnifying instrument, 10 x and 20x and 40 x eminent. The quantities of refined cells were in various gatherings, the count was 0,75×106, 1.5×106, and 3×106 appeared as A, B, and C, separately.

3.3. Growth curve:

At a wavelength between 570 and 590 nm with 560 nm wavelength. on plate reader of ELISA. results calculated within the linear range of the assay. We decided the normal qualities from triplicate readings and subtract the normal incentive for the clear. Plot absorbance against number of cells/ml. The development qualities of a cell line can be dictated by the age of a development bend. This is developed from tests taken at interims all through the development cycle. These stages are trademark for every cell line and are significant when planning routine subculture and experiment protocol, as cell natural chemistry changes significantly during each stage.

3.4. HBVsvp Concentration:

After propagation of HBVsvp containing supernatant, poly Ethelene glycol protocol was used for concentration of HBVsvp in three

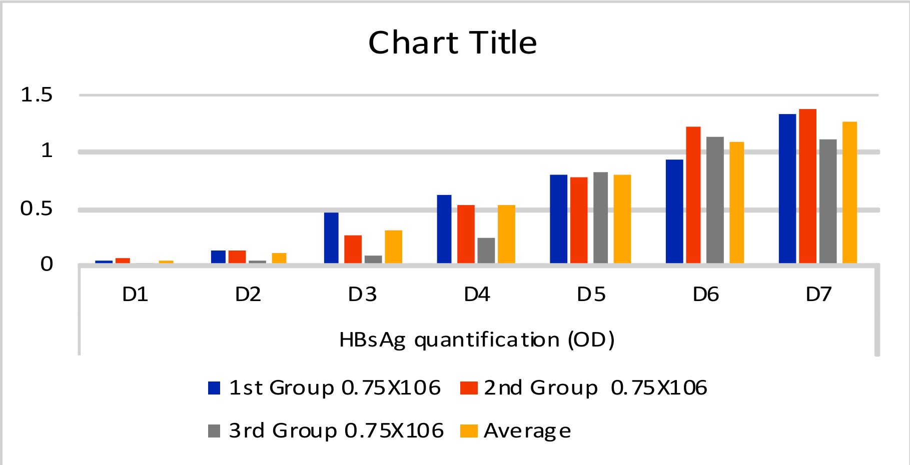


Figure (1): The average of HBsAg quantification (optical density

(A) **(B)** **(C)**

Photograph (2) Showed Morphology of prolonged HepG2.2.15 cells measured by stage differentiate light altered magnifying instrument, 10 x and 20x and 40 x eminent. The quantities of refined cells were in various gatherings, the count was 0,75×106, 1.5×106, and 3×106 appeared as A, B, and C, separately.

groups among 7 days, and quantitative detection of HBsAg were assayed by ELISA, the resulted optical density for samples showed that the highest viral concentration was day 7 concentrated sample (1.708 O.D.) after cultivation of HeoG2.2.15 for seven days compared to concentrated SVP in day one (1.118 O.D).(Table 3; Figure 4).

3.5. Electron Microscopy:

To separate between Dane-particles and SVP of HBV, positive models and supernatant of HepG2.2.15 cell line was investigated by TEM. Our results showed that positive HBV tests exhibited both HBV (Dane-particles) and HBVsvp. Regards to supernatant of HepG2.2.15 cell line, SVP of HBV perceived as it were.

Table 2: Optical density of production of SVP by variable concentration of Hepg2.2.15.

N. of cells	1470	2940	5880	11760	23520	47040	94080	188160	376320	752640
O.D Day 1	0.0035	0.0029	0.021	0.0034	0.00385	0.00425	0.0017	0.00135	-0.0018	-0.00315
O.D Day 2	0.011	0.009	0.0065	0.015	0.0225	0.0955	0.11	0.168	0.396	0.166
O.D Day3	0.014	0.01	0.012	0.0215	0.0505	0.111	0.2275	0.3205	0.753	0.611
O.D Day4	0.021	0.023	0.0145	0.0405	0.0925	0.23	0.423	0.516	0.762	0.632
O.D Day5	0.019	0.026	0.016	0.052	0.1285	0.633	0.7555	0.754	0.7965	0.9355
O.D Day6	0.028	0.0365	0.0115	0.097	0.2265	0.7045	0.7645	0.745	0.6855	0.7215
O.D Day7	0.035	0.0525	0.014	0.1545	0.4445	0.7235	0.7855	0.72	0.7085	0.7245
O.D Day8	0.038	0.056	0.0165	0.397	0.663	0.736	0.74	0.7715	0.707	0.684

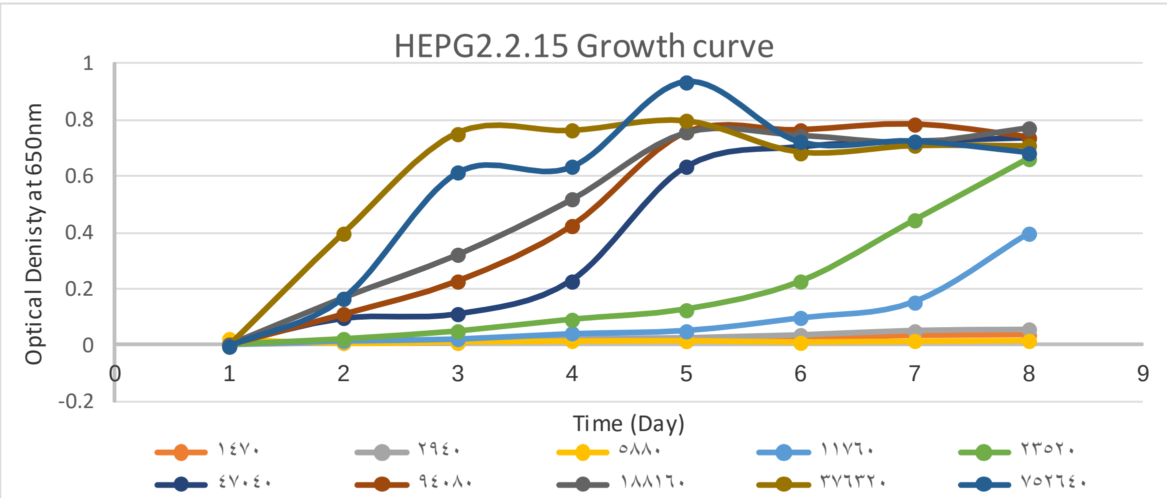


Figure (3): Optical density of production of SVP by variable concentration of Hepg2.2.15

Table (3) HBsAg concentration measured with ELISA before and after concentration

Group	HBsAg optical density Quantification	
	Before concentration	**After concentration**
Day 1	0.028	0.509
Day 2	0.092	1.118
Day 3	0.306	1.2077
Day 4	0.5103	1.6227
Day 5	0.79	1.906
Day 6	1.0783	1.945
Day 7	1.260	1.708

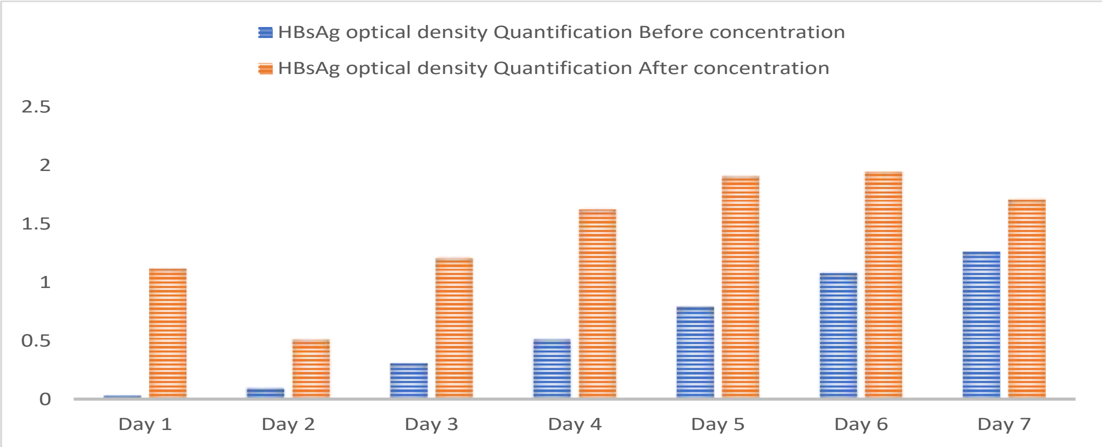
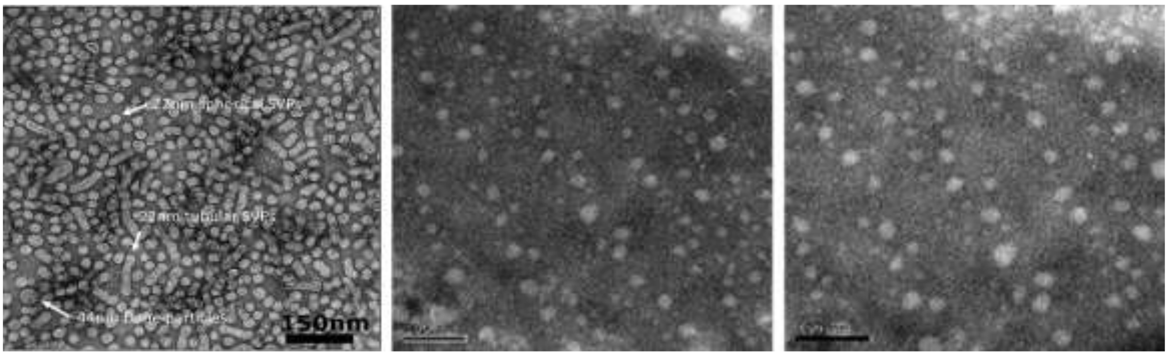


Figure (4)HBsAg concentration measured with ELISA before and after concentration.

(A) **(b)** **(C)**



Photograph (5) Showed Photos of HBV circular SVPs (A) Negative recoloring picture of purified local round SVPs. Each of the 3 sorts of HBV particles are shown. (B) Purified Native SVP(C)= 42 nm HBV (Dane-like molecule)

Photograph (5) Showed Photos of HBV circular SVPs (A) Negative recoloring picture of purified local round SVPs. Each of the 3 sorts of HBV particles are shown. (B) Purified Native SVP(C)= 42 nm HBV (Dane-like molecule)

4. DISCUSSION

About 1 million deaths per year and more than 250 million chronic infections characterize the hepatitis B virus (HBV) as one of the most successful pathogens and global threat to human health. This is especially remarkable as HBV, an enveloped DNA virus, contains a genome of only about 3 kb in size—one of the smallest viral genomes known [17,18]One distinct feature of HBV's life cycle is the production and release of various incomplete and non-infectious particles in addition to the mature virions. Besides genome-free virions, RNA-containing virions, naked NCs, and empty subviral envelope particles (SVPs) are produced consisting of only the viral envelope proteins [19-21].

The wealth of the traditional HBsAg circular and fibers in the blood of HBV contaminated patients enormously encouraged the disclosure of HBV even previously the genome organization[6]. HBV envelope proteins have three forms, called enormous (L), center (M), and little (S), In which S is overwhelming in the two virions and HBsAg particles and L is generally advanced in virions and fibers and scarcely distinguishable in circular[6].

Immune Therapeutic vaccination is another approach that has been employed to break tolerance and stimulate T-cell immune responses in chronic HBV carriers. Immunization with recombinant HBsAg particles from transgenic mice expressing either HBsAg alone or replicating the virus resulted in marked reduction in serum HBsAg levels, loss of HBeAg or even development of anti-HBs [22].

Because of the importance of SVP in the present study, the subviral particles discharged from incorporated HepG2.2.15 were portrayed in vitro using complete Williams medium E including Heat inactivated fetal bovine serum (FBS) that the different thing in our work, insulin and hydrocortisone.

In the current examination HBVsvp were created and described for a few reasons. The principal reason is that they are not irresistible and can along these lines be viewed as more secure in taking care of in concurrence with past examinations[23].Even if irresistible particles found in the HepG2.2.15 supernatant, it's extremely low ratio (1:100.000–1.000.000) and totally could be cleared. Interestingly, different outcomes propose that the cell lines of hepatoma able to deliver irresistible infection when transfect with the genome of virus[24].There is a second significant purpose behind picking HBVsvp for creation and portrayal that they comprise of two genuine basic proteins of hepatitis B virus these proteins presented in a characteristic manner, having equivalent proteins of HBV virion. The Sup Viral Particles segregated from HepG2.2.15 considered more characterized than Sup Viral Particlesharvested from the sera of people infected with the virus, which used historically and recently because the SVP obtained from the sera of infectedpatient may likewise consist of a range of antibodies related to the host either blended in with the SVP or even straightforwardly appended to the SVP [23].

Since the patients delivering SVP are incessantly tainted, the hereditary organization of SVP will be blended, with an assortment of mutant structures inagreement with previous investigation [23]. Whiledisagreementwith [4]which used confined circular SVPs from HBV transporters' sera and decided their 3D structure at the goals of ∼30 å by cryo-electron microscopy (cryo-EM) single-molecule remaking.

All in all, there are important motivations to deliver and portray HBVsvp that accessible by cell line HepG2.2.15 in perfect concentration and furthermore, they could be created for the two genotypes and serotypes. These are with a clinical importance, since it is possible to immunized people prior to infection, as non-responders to the existing vaccine in agreement with previous investigations [23,25] and treatment of chronic patients depending on unique immune therapy. Our present study demonstrates developmentof cell line HepG2.2.15 in complete media (Williams medium E) remembering heat for initiated fetal cow-like serum, insulin and hydrocortisone bringing about normal creation and articulation high measures of discharged SVP in the supernatant in agreement with [23].insufficient information known about HBV morphogenesis. It may be a result of low pace of HBV creation in these cells,as it has been surveyed that an individual hepatocyte discharges just 1 to 10 diseases for consistently as the beneficial period of contamination in human body [23].

In the present study results of electron microscopy displayed that cell line of HepG2.2.15incorporated with genome of HBV had the option to deliver circular and filamentous Subviral particles. Harvesting of SVPgave some intriguing bits of knowledge. (i) according to our exploratory conditions, there were overabundance folds of SVP delivered may reversed to adding heat inactivated Fetal bovine serum. (ii) Envelope proteins of SVP were (22-25-nm) round to fibers particles. (iv) The human immunity cooperates in the pathogenesis of hepatitis; however, it isn't clear the instrument for this connection. The coordinated HepG2.2.15 cells can possibly use as an in vitro model framework for the examination of the collaboration of explicit cytotoxic T lymphocytes and cytolytic antibodies with diseased liver cells. conclusively, our outcomes demonstrated the refined of HepG2.2.15 in completemedia (Williams medium E) remembering heat for in activated fetal bovine serum, insulin and hydrocortisone brings about regular creation and articulation high measures of suspended SVP in the supernatant.

5. CONCLUSION:

These findings shed light on an important technique used in production of huge number of HBVSVP which is important advance in HBV irresistible cycle, as the gathering of HBV sub viral particles potency connected to pathogenesis of virus in order to enhance overcome of HBV infection.

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Author Disclosure Statement

The authors report no conflicts of interest in this work. The authors have no competing financial interests and are solely responsible for the experimental designs and data analysis.

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الاتجاهات المتقدمة لإنتاج جزيئات فيروس التهاب الكبد (ب) باستخدام تقنيات مختلفة لتعزيز التغلب على عدوى فيروس التهاب الكبد الوبائي (ب)

محمد عبد الفتاح سليم[1] ، عادل عبد الباسط[1] ، محمد منصور سعد فرج[1] ، محمد طارق منصور[2]

1- قسم النبات ، كلية العلوم ، جامعة الازهر
2- معهد الاورام جامعة القاهرة

الملخص العربي:

عدوى فيروس التهاب الكبد المزمن (ب)هي واحدة من عوامل الخطر الرئيسية لالتهاب الكبد المزمن، تليف الكبد، تليف الكبد وسرطان الكبد الخلوي وهي مشكله صحية عالميه رئيسية حتى مع استخدام اللقاح والشفاء الذاتي في معظم الحالات.يحتوي فيروس التهاب الكبد على أنواع مختلفة من الجسيمات، أي الجسيمات الكروية والأنبوبية الفرعية ذات 22 نانومتر. كل من الجسيمات الكروية والانبوبية تكون غير معدية ولكنها قد تمثل مناعة قوية. وكان الهدف الرئيسي من هذه الدراسة الحصول على أفضل الطرق لإنتاج جزيئات الفيروس(ب) بأنواعها والتي يتم افرازها من الخلايا المخلقة معمليا (خلايا كبديه 2-2-15) داخل المعملوذلك بغرض الاستفادة منها فيما بعض في احدى طرق العلاج المناعي لفيروس بي. ولتحقيق ذلك الهدف تم زراعة الخلايا المخلقة معمليا (خلايا كبديه 2-2-15) لإنتاج ومن ثم تركيز جزيئات فيروس (ب) بنوعيها باستخدام الميديا المخصصة وكذلك البولي ايثيلين جليكول بغرض التركيز. ثم بعد لك تم فحص الجزيئات الفيروسية بالطرق السير ولوجيه وكذلك باستخدام الميكروسكوب الالكتروني. نتائجنا تشير الى ان زراعة تلك الخلايا في وسط متكامل يحتوي على السيرم المسخن من قبل عند درجة حرارة 56 بنسبة 10%بالإضافة الي الانسولين والهيدروكورتيزون أدى في النهاية الى انتاج كميات كبيره من الجزيئات الفيروسية لفيروس التهاب الكبد الوبائي (ب). ولمعرفة الشكل الظاهري والتركيب الخاص بتلك الجزيئات الفيروسية تم استخدام الميكروسكوب الالكتروني مما اظهر الشكل الخيطي والكروي لتلك الجزيئات الفيروسية والتي تتشابه مع الشكل الظاهري لفيروس (ب) ولكنها تختلف في الحجم والتركيب.

هذه الدراسة القت الضوء على تقنيات مهمة مستخدمة في انتاج كميات هائلة من تلك الجزيئات الفيروسية والتي لها دور كبير جدا في عملية حدوث العدوى بفيروس س(ب) لذلك فان المضي قدما في انتاج كميات هائلة من تلك الجزيئات يساعدنا في فهم أكثر للمراحل التي يمر بها الفيروس مما يساهم في معرفة ادق تفاصيل الاصابة ومن ثم المساعدة في التغلب على العدوى بفيروس (ب).